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CHRISTIAAN HUYGHENS AND THE WAVE THEORY OF LIGHT

By Sir C. V. Raman

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1. Introduction

The world, as we perceive it, is pictured for us by the rays of light which proceed from each point of the objects around us and form images of these objects on the retinæ of our eyes—a statement which needs no amendment even when the aid of optical instruments such as the telescope or the microscope is invoked to enlarge our powers of vision. To put it a little differently, the principles of geometrical optics suffice to describe the behaviour of light as commonly experienced, *viz.*, that the rays of light are propagated in straight lines; that the angles of incidence and of reflection are equal; and that in refraction the rays of light are bent according to the law of sines. In his celebrated *Traite de la Lumiere* published in the year 1690, Christiaan Huyghens showed that these facts of experience are consistent with the hypothesis that light is in the nature of wave-motion propagated through space and can indeed be satisfactorily explained on the basis of that hypothesis. The treatise of Huyghens contains much other material of importance; a perusal of it leaves on the mind of the reader the impression that it is a masterpiece of scientific thought and exposition which possesses an enduring value and interest.

In connection with some experimental investigations on the diffraction of light undertaken by the present author—the results of which will soon be published in these *Proceedings*—the need was felt for a careful study of the original ideas of Huyghens. The task was made much easier by using the literal translation of his treatise from the original French into English by Sylvanus P. Thompson published by the Chicago University Press in the year 1912. The results of the study were surprising; it emerged that the ideas of Huyghens were not fully or even correctly understood by later writers. This misunderstanding has had some far-reaching consequences. Especially in regard to the so-called "Principle of Huyghens" do we find that later writers have chosen a path for which there is no warrant in the writings of Huyghens. In view of these circumstances, it has appeared desirable to put forward a clear exposition of the ideas of Huyghens and supplement the same

by a critical examination of the writings of later authors which claim to be based on those ideas.

2. THE NATURE OF LIGHT

In the first few pages of his book Huyghens set out the considerations which led him to infer that light is in the nature of a movement which spreads into space in all directions from a luminous source. He remarks that the terrestrial sources which are observed to emit light, such as fire or flame evidently contain bodies in rapid motion. Then again, when sunlight is collected by a concave mirror and concentrated on material objects, it has the same effects as fire, *viz.*, it disunites the particles of those objects. It is therefore natural to suppose that light is itself some kind of motion and that the sensation of light is excited when such movement is communicated to the nerves at the back of the human eye. Huyghens also remarks on the extreme speed with which light spreads on every side and on the circumstance that when light comes from different regions, even from those directly opposite each other, the rays traverse one another without hindrance. The facts indicate that light is a movement transmitted through space and not a transport of matter which reaches the eye from the source of light.

To account for the very high velocity of propagation of light—known from the observations of Römer on the eclipses of Jupiter's satellites—and the fact that light can pass through empty space, Huyghens proposed a physical picture of the ætherial medium which could explain its power to transmit waves with such high velocity. He suggested that the æther of space consists of an immense number of extremely small and extremely hard spherical particles in close contact with each other. Experiments on the percussion of elastic solid spheres on each other show that a medium of the nature postulated could propagate waves simultaneously in all directions with high velocity and in such manner that waves travelling in different directions at one and the same time would not hinder each other's progress. Huyghens further recognized that every luminous object would necessarily contain an immense number of centres emitting light and that from each of such centres thousands of waves might emerge in the smallest imaginable time; he pointed out that these considerations would make it easier to understand, why in spite of the enfeeblement of the individual waves by their spread through immense distances, the light of the distant stars continues to be perceptible to human eyes.

3. THE RECTILINEAR PROPAGATION OF LIGHT

The mechanical model of the æther proposed by Huyghens to account for the propagation of waves of light through it also enabled him to give a

simple and satisfactory explanation of why light travels out from the original source in straight lines. Considering the medium which transmits light to be composed of an immense number of very small and very hard spherical particles in close contact with each other, it follows that each of these particles when it is displaced from its position by the passage over it of the parent wave sent out from the original source would itself function as the source of a wave which spreads out from it in all directions. The particular or partial waves of this nature recognized by Huyghens in his argument would be as numerous as the total number of the individual particles of æther contained in a sphere drawn with the source of the primary wave as centre and the distance to which it has travelled out as the radius. It is evident, also, that these partial waves, though present in enormous numbers within the volume of the sphere, would individually be of excessively feeble force. In theory, they are assumed to travel out from their respective centres in all directions but actually, by reason of their excessive feebleness the effect of all the partial waves may be totally ignored except of those which arrive together at the same instant, in other words, *simultaneously*, at the point of observation. For, in the latter case, their effects would be superposed and would add up to give an observable result. Such simultaneity in arrival and consequent superposition of effects would be possible only in the case of those partial waves which originate at points in the medium between the primary source of light and the point of observation which lie on the straight line joining them. The summation of the partial waves originating at such points and reaching the point of observation at the same instant would produce the luminous effect there observed. Thus the light which reaches the point of observation from the original source may be considered as having travelled out along the straight line joining the two points.

4. The Reflection and Refraction of Light

In the second and third chapters of his treatise, Huyghens considered the phenomena which arise when light travelling in one medium reaches the boundary which separates it from another. In considering those phenomena, various physical questions arise and are discussed by him, as for example, why some materials are transparent to light and others are opaque, why the velocity of light in a material medium should differ from that in empty space and what the actual configuration of the boundary of the separation between two media is or can be.

The explanation of reflection and refraction in terms of the wave hypothesis is based on an idea which Huyghens found himself compelled to introduce, *viz.*, *that the elements of the area* of the boundary of separation between

the two media act as sources of partial waves which travel out respectively into the two media. The partial waves returned from the boundary into the first medium build up the regularly reflected wave, while those travelling into the second medium give the refracted wave.

A simple geometric construction based on the foregoing ideas enabled Huyghens to explain the familiar law of reflection of light. Likewise, assuming the second medium to be transparent and that the velocity of light in it differs from that in the first, a similar geometric construction led him to the result that the sine of the angle of incidence and the sine of the angle of refraction bear to each other a constant ratio which is the same as the ratio of the velocities of light in the two media. The phenomena of the total internal reflection of light also found a satisfactory explanation. Huyghens showed further that his construction leads directly to Fermat's principle of minimum time for the passage of light from one point to another when the two points are in different media.

5. THE WAVE-OPTICS OF HUYGHENS

Before proceeding to comment on the writing of later authors on the work of Huyghens, we may usefully here summarise the basic concepts of his theory. Huyghens put forward and sought to establish the proposition that when a wave of light diverges from its source, every small portion of the wave is capable of propagating itself independently with the same velocity as the rest of it; in an isotropic medium, the direction of such propagation is the wave-normal and hence this is also the direction of the ray in the sense of geometrical optics. The same idea forms the basis of Huyghens' explanation of the reflection and refraction of light. When the elements of area of an advancing wave-front reach the boundary between two media, each such element gives rise, respectively in the two media, to the elements of area in the reflected and refracted waves. These latter advance normally to themselves in such a direction that they can join up and form continuous wave-fronts. The geometric constructions employed by Huyghens enable these requirements to be satisfied. The propagation of light in an inhomogeneous medium considered in the fourth chapter of Huyghens' treatise can also be very simply dealt with on the same basis. The elements of area of the wave-front in such a medium advance normally to themselves with the velocity appropriate to their positions in the medium. As they advance, they join up to form new wave-fronts which are orthogonal to the path of the light-rays in the medium.

Later writers have criticised the arguments employed by Huyghens in his treatise. One remark which is often made is that the theory of Huyghens

would result in his wave-fronts moving backwards as well as forwards and that he had given no explanation for the absence of backward propagation. But this criticism is not justified and is itself based on a misunderstanding. Huyghens was concerned with the behaviour of an *advancing* wave-front in a homogeneous medium. The partial waves which in his theory give the observed light intensity by their superposition are those which diverge from points lying on the straight line between the source and the observer; in order to reach the observer simultaneously they should all move *away* from the source and *towards* the point of observation, in other words move *forwards* towards the observer. The possibility of backward propagation is thus ruled out completely.

Another criticism which has frequently been advanced is that the theory of Huyghens is based on an arbitrary assumption, *viz.*, that only along the envelope of his partial waves would there by any observable intensity of light. This criticism is also based on a misunderstanding. It should be remembered that Huyghens was unaware that the waves of light are periodic disturbances having a definite wave-length. He assumed that light consists of *individual* waves which diverge in all directions from the original source and the partial waves contemplated in his theory would therefore also be of the same nature. The build-up of a finite intensity from the superposition of a very large number of such waves, each of which is extremely feeble, would accordingly be possible only if they arrive *simultaneously* at the point of observation. The diagram appearing in the first chapter of Huyghens' treatise is intended to assist the reader to appreciate the arguments set out in the text; *viz.*, at *each* point on the wave-front a great number of partial waves arrive *simultaneously* and build up the intensity at that point, while the entire wave may be itself considered as made up of a great number of elementary areas at which the light-intensity has thus been built up. In the later chapters in which Huyghens' theories of reflection and refraction and of the propagation of light in an inhomogeneous medium are expounded, the diagrams are intended to exhibit how the complete wave-front arising from these processes is built up out of its elementary parts or areas. Here again, the final result is an individual wave, and it may therefore be correctly described as the envelope of the partial waves which co-operate in building it up.

6. The Partial Waves of Huyghens

Since the concept of partial waves introduced by Huyghens in his treatise has played an important role in physical optics, it is appropriate that we consider it here in some detail. Though the words appear in several chapters

of his treatise, it should be remarked that they do not have the same significance in each case. In the first chapter which seeks to explain the rectilinear propagation of light, the partial waves arise as a consequence of the assumed discrete structure of the luminiferous medium; each particle in the medium is regarded as a source of such waves. In the second and third chapters, the partial waves are assumed to arise when the primary wave reaches the boundary separating the two media with different properties. The elements of area of the boundary are here regarded as the source of partial waves. Since they travel with different velocities, they are distinct from each other in the two media. In the fourth chapter which deals with the propagation of light in inhomogeneous media, the partial waves are assumed to diverge from the elements of area of the advancing wave-front in such a medium.

If the luminiferous medium were empty space, the assumption that it consists of discrete particles which can function as emitters of partial waves would be difficult to justify. In the case of material media, however, there is good reason for assuming that the discrete atoms of which they are composed could function as sources of secondary or partial waves. Even so, however, these partial waves would reinforce each other in the direction of propagation of the primary wave and merge with it, while in other directions they would interfere and cancel out each other's effects. Thus, they would, in all cases, cease to be observable. Accordingly, the notion of partial waves can, in such circumstances, be regarded only as hypothetical or virtual and not as an observable or physical reality. The same remarks would also be applicable in regard to the propagation of light in a medium which is inhomogeneous. Indeed, as already remarked, this particular case could be dealt with in a very simple manner without making any use of the concept of partial waves. Thus, finally, we are left with the phenomena arising from the incidence of light on the boundary between two material media. Huyghens' construction explains the geometric laws of reflection and refraction in so natural and convincing a fashion that it is difficult to resist the conclusion that his concept of partial waves is well-grounded and is a physical reality in these particular cases.

7. THE SO-CALLED PRINCIPLE OF HUYGHENS

It will be evident from what has been said above that the ideas of Huyghens were not correctly understood or appreciated by later writers. It is not surprising therefore that the whole of the vast literature which was subsequently published and which claims to base itself on the ideas of Huyghens, in reality proceeds on a different basis altogether. This is evident from the fact that the mathematicians whose objective was to develop a "Rigorous

Formulation of the Principle of Huyghens" concerned themselves with precisely the case in which Huyghens' concept of partial waves has no physical meaning or justification, namely the undisturbed propagation of waves from a source situated in a structureless and uniform continuum.

The well-known formula developed by Kirchhoff is an illustration of the foregoing remarks. Here, the disturbance due to the source at the point of observation is expressed as an integral taken over the area of a closed surface within which the point of observation is included but not the source. Each elementary area of the surface appears in the formula as a source from waves diverge with amplitudes which vary with the direction of emission. The line joining the source and the point of observation is also the direction of maximum amplitude for the waves radiated by the element of area which lies on that line *between* them, and of zero amplitude for an element of area which also lies on the same line but on the *opposite side*. Kirchhoff's formula as actually developed refers to the case of sound-waves, and the attempts made to extend it to the case of light have not met with success. But our present concern is not with the mathematics of the formula but with the physics of the subject. The association of the formula with the name of Huyghens—honoured as the founder of the wave-theory of light—has naturally disposed whole generations of physicists to look upon it with favour. It has, however, been made clear by the foregoing remarks that Kirchhoff's approach to the subject is quite different from that of Huyghens. We have, therefore, to ask ourselves: Is Kirchhoff's formula really meaningful? Has it any claim to validity or acceptance considered from the standpoint of optical theory? We shall proceed to consider these questions.

As has already been remarked, one of Huyghens' striking successes is his explanation of the geometric laws of reflection and refraction. His concept of partial waves takes its clearest and most acceptable form in this case, *viz.*, that each element of area of the physical boundary acts as a source of partial waves. Since these move with different velocities in the two media, they should be considered as distinct. In other words, the partial waves in each medium are hemispherical, and it becomes a meaningful physical problem to determine the dependence of the amplitude of the waves with direction on the surface of these hemispheres. It would presumably be a maximum in the direction of the normal to the boundary and zero in directions parallel to the boundary. On the other hand, the very generality of Kirchhoff's formula indicates that it has no physical validity or significance. For, it is not possible to discover or assign any reason why an element of area set at an arbitrary orientation in a continuous structureless medium

should function as a source of secondary waves with specific features related to that orientation. If the concept of partial or secondary waves is at all to be meaningful, the waves should have a physically recognizable origin, *e.g.*, a local discontinuity in physical properties. In its absence, the formula ceases to have any physical content. Kirchhoff's formula thus reveals itself to be a mathematical abstraction which is not relevant or valid in relation to the actual problems of physical optics.

8. SUMMARY

The " Principle of Huyghens " has played an important role in physical optics and especially in the explanation of the phenomena of the diffraction of light. An examination of Huyghen's own ideas as expounded in his original treatise is therefore of interest and is undertaken in the present memoir. It emerges that the particular form given to the " Principle " by mathematical analysts, notably Kirchhoff, does not find any support or warrant in the writings of Huyghens. The concept of " partial waves " introduced by Huyghens in his treatise is based on specific physical considerations. These are totally lacking in relation to the free and uninterrupted propagation of waves in a continuous structureless medium. The latter case is considered in Kirchhoff's theory and it follows that the Kirchhoff formula is a mathematical abstraction which has no relevance or validity in relation to the actual problems of physical optics.

STUDIES IN THE FORMATION OF HETEROCYCLIC RINGS CONTAINING NITROGEN

Part VII. Condensation of 4-Nitro-*o*-Phenylenediamine with Aromatic Aldehydes

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Pinnow and Wiskott[1] reported the formation of mono and disubstituted benziminazoles in the condensation of 4-nitro-*o*-phenylenediamine hydrochloride with two moles of benzaldehyde and *m*-nitro benzaldehyde. However, they could not succeed in their attempts to fix the position of nitro group (5 or 6) in the disubstituted benziminazoles; this has been recently established by the present authors[2] to be 6 adopting definite synthetic methods. Feitelson and collaborators[3] carried out the condensation of the diamine with one mole of *p*-nitrobenzaldehyde by refluxing in acetic acid for 5 hours, and obtained the corresponding mono and disubstituted benziminazoles, the former being formed in minute amounts. They did neither mention about the isomerism nor made any attempts to determine the position of nitro group in the disubstituted benziminazole, but named it as 1-(*p*-nitrobenzyl)-2-(*p*-nitrophenyl)-5-nitrobenziminazole. Perhaps, these workers did not pay attention to this problem.

Condensation of 4-nitro-*o*-phenylenediamine with seventeen aromatic aldehydes (other than benzaldehyde[2]) has now been investigated with a view to studying the influence of substituents in the aldehydes as well as of the nitro group of the diamine on the nature and relative yields of the reaction products. The condensations have been carried out following the modified Hinsberg's procedure,[4] except that hot acetic acid has been used on account of the low solubility of the diamine in the cold acid. It has been found that the mono and disubstituted benziminazoles formed in the condensations with aldehydes other than hydroxy aldehydes could be conveniently separated making use of 5% potassium hydroxide solution in which the monosubstituted benziminazoles are preferentially soluble.

In the case of a few aldehydes, dianils (II) or monoanils (I) have been isolated along with the corresponding benziminazoles. 5-Nitrosalicylaldehyde gave the dianil only. The anils isolated have been identified by cyclising them to the corresponding benziminazoles and comparing with those obtained

by direct condensation. These facts leave no doubt as to the Schiff's bases being intermediates in the formation of benziminazoles. The results, presented in Table I, thus lend strong support to the step-wise mechanism proposed earlier.[4]

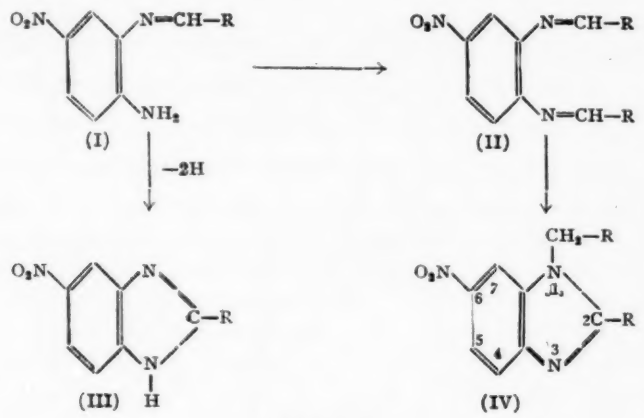

TABLE I

Products of condensation of 4-*nitro*-o-*phenylenediamine with R.CHO*

No.	R	% Yield of			
		I	II	III	IV
1	*p*-tolyl	..	..	18	81
2	*o*-hydroxyphenyl	..	..	24	71
3	*m*-hydroxyphenyl	..	..	..	84
4	*p*-hydroxyphenyl	..	..	35	59
5	*m*-methoxy-*p*-hydroxyphenyl	..	..	26	57
6	3:4-methylenedioxyphenyl	..	..	15	80
7	*p*-methoxyphenyl	..	..	28	66
8	*o*-chlorophenyl	..	44	43*[5]	..
9	*p*-chlorophenyl	..	..	35*[5]	63
10	2:4-dichlorophenyl	..	..	50	..
11	3:4-dichlorophenyl	..	45	30	22
12	*o*-nitrophenyl	..	12	54	20
13	*m*-nitrophenyl	..	43	43*[1]	8*[1]
14	*p*-nitrophenyl	..	53*[6]	10*[3,6]	23*[3]
15	2-hydroxy-5-nitrophenyl	..	61	..	..
16	*p*-dimethylaminophenyl	..	..	95	..
17	1-naphthyl	..	..	25	74

* Compounds reported in literature.

Except in the case of *m*-hydroxybenzaldehyde, all other hydroxy aldehydes yielded considerable amounts of monosubstituted benziminazoles from their reaction with the diamine. Surprisingly enough, the diamine afforded nearly quantitative yield of the mono derivative with *p*-dimethylaminobenzaldehyde. A plausible explanation for the formation of these monosubstituted benziminazoles in greater proportion from aldehydes containing electron-releasing groups may be attributed to the predominating influence of the nitro group of the diamine. Aldehydes containing electron-withdrawing groups gave comparatively larger amounts of monosubstituted benziminazoles and or monoanils, probably due to the combined influence of the aldehyde substituent and the nitro group of the diamine. It is therefore obvious that in these condensations, after the formation of monoanil (I), further condensation of (I) to form a disubstituted benziminazoles (IV) is not as much favoured as its undergoing oxidative cyclisation to yield the monosubstituted benziminazole (III).

From these results it may be inferred that the presence of a powerful anionoid or cationoid substituent in the diamine can alter or modify the influence of the substituent group in the aldehyde with which it is reacting, and the net effect of both may ultimately determine the nature and yields of the reaction products.

Experimental

All m.p.'s are uncorrected. The micro-analyses were carried out by one of the authors (C. V. R.).

General procedure for the condensations.—4-Nitro-*o*-phenylenediamine (one mole), dissolved in the minimum quantity of boiling acetic acid, was mixed with a similar solution of aromatic aldehyde (two moles) and set aside for one hour with occasional stirring. Any solid that separated from the solution, or after cooling was filtered; the filtrate was added to excess of crushed ice with vigorous stirring and the substance that separated was filtered. The dilute acid filtrate gave no appreciable product on making it ammoniacal. The substances thus isolated were separately triturated with 5% potassium hydroxide solution and the alkali soluble portion was isolated by precipitation with acetic acid. The alkali soluble and insoluble compounds were purified and characterised.

The various anils isolated in these condensations were cyclised to the corresponding benziminazoles by refluxing in acetic acid for two hours. However, the anil from 5-nitrosalicylaldehyde required a period of 12 hour s

TABLE II

Properties and analytical data of the new compounds

No.	Name of the Compound	Colour and crystalline shape	m.p. (° C.)	Formula	% of N Found	% of N Required
1	1-(*p*-methylbenzyl)-2-(*p*-methylphenyl)-6-nitro benziminazole[a]	light yellow prismatic rods	160	$C_{22}H_{19}N_3O_2$	11·7	11·8
2	2-(*p*-methylphenyl)-5 (or 6)-nitro benziminazole[a]	colourless plates	215	$C_{14}H_{11}N_3O_2$	16·2	16·6
3	1-(*o*-hydroxybenzyl)-2-(*o*-hydroxyphenyl)-6-nitro benziminazole[b]	pale yellow rectangular rods	211	$C_{20}H_{15}N_3O_4$	11·0	11·6
4	2-(*o*-hydroxyphenyl)-5 (or 6)-nitro benziminazole[b]	pale yellow needles	297	$C_{13}H_9N_3O_2$	16·6	16·5
5	1-(*m*-hydroxybenzyl)-2-(*m*-hydroxyphenyl)-6-nitro benziminazole[a]	pale yellow plates	276	$C_{20}H_{15}N_3O_4$	11·7	11·6
6	1-(*p*-hydroxybenzyl)-2-(*p*-hydroxyphenyl)-6-nitro benziminazole[a]	pale yellow rectangular rods	245	$C_{20}H_{15}N_3O_4$	11·0	11·6
7	2-(*p*-hydroxyphenyl)-5 (or 6)-nitro benziminazole[a]	yellow microcrystalline	328*	$C_{13}H_9N_3O_3$	15·9	16·5
8	1-(*m*-methoxy-*p*-hydroxybenzyl)-2-(*m*-methoxy-*p*-hydroxyphenyl)-6-nitro benziminazole[a]	orange rectangular plates	216	$C_{22}H_{19}N_3O_6$	10·1	10·0
9	2-(*m*-methoxy-*p*-hydroxyphenyl)-5 (or 6)-nitro benziminazole[a]	yellow plates	283*	$C_{14}H_{11}N_3O_4$	14·5	14·7
10	1-(3 : 4-methylenedioxybenzyl)-2-(3 : 4-methylenedioxyphenyl)-6-nitro benziminazole[d]	tiny yellow rods	224	$C_{22}H_{15}N_3O_6$	9·8	10·1
11	2-(3 : 4-methylenedioxyphenyl)-5 (or 6)-nitro benziminazole[a]	yellow rectangular rods	248	$C_{14}H_9N_3O_4$	14·3	14·8
12	1-(*p*-methoxybenzyl)-2-(*p*-methoxyphenyl)-6-nitro benziminazole[a]	light yellow prismatic rods	132	$C_{22}H_{19}N_3O_4$	10·3	10·8
13	2-(*p*-methoxyphenyl)-5 (or 6)-nitro benziminazole[a]	yellow needles	234	$C_{14}H_{11}N_3O_3$	15·6	15·6
14	N : N′-*di*-(*o*-chlorobenzylidene)-4-nitro-*o*-phenylenediamine[a]	red rectangular plates	175	$C_{20}H_{13}N_3O_2Cl_2$	11·0	10·6

13	2-(*p*-methoxyphenyl)-5 (or 6)-nitro benziminazole[a]					
14	N: N′-*di*-(*o*-chlorobenzylidene)-4-nitro-*o*-phenylenediamine[a]	red rectangular plates	175	$C_{20}H_{13}N_3O_2Cl_2$	11·0	10·6
15	1-(*p*-chlorobenzyl)-2-(*p*-chlorophenyl)-6-nitro benziminazole[e]	colourless needles	208	$C_{20}H_{13}N_3O_2Cl_2$	10·8	10·6
16	2-(2: 4-dichlorophenyl)-5 (or 6)-nitro benziminazole[a]	pale yellow needles	242	$C_{13}H_7N_3O_2Cl_2$	13·8	13·6
17	N: N′-*di*-(3: 4-dichlorobenzylidene)-4-nitro-*o*-phenylenediamine[e]	dark red rhombic rods	166	$C_{20}H_{11}N_3O_2Cl_4$	9·3	9·0
18	1-(3: 4-dichlorobenzyl)-2-(3: 4-dichlorophenyl)-6-nitrobenzimina-zole[e]	transparent plates	223	$C_{20}H_{11}N_3O_2Cl_4$	9·5	9·0
19	2-(3: 4-dichlorophenyl)-5 (or 6)-nitro benziminazole[e]	yellow needles	295	$C_{13}H_7N_3O_2Cl_2$	13·2	13·6
20	N-(*o*-nitrobenzylidene)-4-nitro-*o*-phenylenediamine[a]	orange rectangular rods	218	$C_{13}H_{10}N_4O_4$	19·0	19·6
21	1-(*o*-nitrobenzyl)-2-(*o*-nitrophenyl) -6-nitro benziminazole[e]	orange yellow prisms	270	$C_{20}H_{13}N_5O_6$	17·2	16·7
22	2-(*o*-nitrophenyl)-5 (or 6)-nitro benziminazole[a]	transparent rectangular plates	222	$C_{13}H_8N_4O_4$	19·5	19·7
23	N: N′-*di* (*m*-nitrobenzylidene)-4-nitro-*o*-phenylenediamine[a]	deep red needles	188†	$C_{20}H_{13}N_5O_6$	16·5	16·7
24	N: N′-*di*-(2-hydroxy-5-nitro benzylidene)-4-nitro-*o*-phenylenediamine[f]	orange yellow needles	238	$C_{20}H_{13}N_5O_8$	16·0	15·5
25	1-(2-hydroxy-5-nitrobenzyl)-2-(2-hydroxy-5-nitrophenyl)-6-nitro benziminazole[g]	yellow needles	315*	$C_{20}H_{13}N_5O_8$	16·1	15·5
26	2-(*p*-dimethylaminophenyl)-5 (or 6)-nitro benziminazole[a]	orange yellow rectangular rods	220	$C_{15}H_{14}N_4O_2$	19·5	19·9
27	1-(1-naphtyl methyl)-2-(1-naphthyl)-6-nitro benziminazole[e]	yellow plates	218	$C_{28}H_{19}N_3O_2$	9·5	9·8
28	2-(1-naphthyl)-5 (or 6)-nitro benziminazole[a]	rectangular plates	226	$C_{17}H_{11}N_3O_2$	14·0	14·5

Solvents of recrystallisation: [a] alcohol; [b] benzene-alcohol; [c] benzene; [d] ethyl acetate; [e] alcohol-acetone; [f] nitrobenzene; [g] benzene-acetone.

* Decomposition;

† Melting at 188°, resolidifying to an yellow mass by 210°, which melts at 230°.

of refluxing for cyclisation, while attempts to cyclise the anil from *o*-chlorobenzaldehyde were unsuccessful.

Products of condensation.—In the condensation of the diamine with *p*-tolualdehyde, piperonal, and anisaldehyde, no solids separated from the glacial acetic acid solution. The products obtained from the dilute acid solution, on fractionation with alkali, yielded alkali insoluble compounds characterised as disubstituted benziminazoles, and alkali soluble substances identified as monosubstituted benziminazoles.

The products that separated by cooling the acetic acid solution in the *p*-chlorobenzaldehyde and 1-naphthaldehyde reactions were completely soluble in alkali and could be characterised as monosubstituted benziminazoles, while the products obtained from the dilute acid solution were found to be mostly the alkali insoluble disubstituted derivatives.

In the condensation with *o*- and *p*-hydroxybenzaldehydes, the substances that separated by cooling the acetic acid solution were found to be monosubstituted benziminazoles, while the compounds from dilute acid solution were the disubstituted derivatives. No substance was isolated from the acetic acid solution in the case of *m*-hydroxybenzaldehyde, and the compound that separated from the dilute acid solution was found to be disubstituted benziminazole.

No solid separated from the acetic acid solution in the condensation with vanillin, and the product isolated from the dilute acid solution yielded two compounds on fractionation with alcohol. The alcohol insoluble compound was identified as the monosubstituted benziminazole and the alcohol soluble compound as the disubstituted derivative.

The products that separated from the acetic acid solution in 2:4-dichloro and *p*-dimethylamino benzaldehyde reactions were found to be the alkali soluble monosubstituted benziminazoles.

In the condensation with 3:4-dichloro and *m*-nitro benzaldehydes, the products that separated from the acetic acid solution were found to be mixtures of dianils and monosubstituted benziminazoles which could be separated on the basis of the solubility of the latter in alkali; the compounds obtained by diluting the acid filtrates were identified as the disubstituted benziminazoles.

The substances obtained from the acetic acid solution in the *o*- and *p*-nitrobenzaldehyde reactions, on fractionation with alkali, gave alkali soluble monosubstituted benziminazoles, and alkali insoluble products, which on fractional crystallisation from alcohol gave alcohol insoluble monoanils

and alcohol soluble disubstituted benziminazoles. The products that separated by diluting the acid filtrates, in both the cases, were found to be mostly the disubstituted benziminazoles.

In the condensation with *o*-chlorobenzaldehyde, the solid that separated from acetic acid solution on cooling as well as that obtained from dilute acid solution were found to be mixtures of dianil and monosubstituted benziminazole. The compound that separated from acetic acid solution in 5-nitrosalicylaldehyde reaction was found to be the dianil.

The properties and analytical data of the new compounds isolated during this investigation are given in Table II.

Summary

On condensation with aromatic aldehydes 4-nitro-*o*-phenylenediamine has been found to yield mono and disubstituted benziminazoles, as well as mono or dianils in a few cases. The formation of monosubstituted benziminazoles or monoanils in greater proportion in these condensations than in those with methyl and chloro diamines may be attributed to the predominating influence of the nitro group of the diamine. The results provide strong support to the step-wise mechanism for the formation of benziminazoles through the intermediate anils.

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THE PHOTOLYTIC PREPARATION OF URANIUM TETRAFLUORIDE

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(Communicated by Prof. G. N. Ramachandran, F.A.SC.)

Introduction

The photolytic preparation of uranium tetrafluoride was indicated in one of our earlier communication[1] when the utilization of solar energy had not been reported for the isolation of the compound or its complex. This photolytic method not only eliminates the use of foreign substances used for the purpose of reduction but also minimizes many of the operations involved in other processes of preparation. Of all the common compounds of uranium, advantage has been taken of the use of uranyl formate for the readiness with which it undergoes photolysis in the presence of alcohol on exposure to bright sunlight giving uranium (IV) compounds.[2-3] Thus when photolysis is carried out in aqueous hydrofluoric acid medium, the tetrafluoride is precipitated. Use of uranyl nitrate also gives the same compound. When, however, photolysis is carried out in the presence of ammonium bifluoride, in place of hydrofluoric acid the complex NH_4F, UF_4, H_2O is obtained which will be published in due course.

Experimental

All the chemicals used were E. Merck, E.P. or G.R. quality. The formate was prepared from the nitrate. When a saturated solution of uranyl formate or nitrate in 38–40 per cent. of hydrofluoric acid with half its volume of alcohol was exposed to sunlight in a platinum basin, bright green uranium tetrafluoride began to appear in about 15 minutes time. On further exposure, more of the compound was formed and settled down. The supernatant liquid was decanted off, and the precipitate was washed with aqueous hydrofluoric acid. The analysis of the compound was undertaken after it was dried for about 3 hours at 100° C. in an air-oven.

The molecular weight was determined by dissolving a weighed quantity of the compound in 8 N HCl, precipitating uranium as hydrated oxide with NH_4OH and then heating the hydrate oxide, after filtration to U_3O_8 in a

platinum crucible. The filtrate was used for the estimation of fluoride as CaF_2 by Badeeva's[4] method. Uranium (IV) content was estimated by the method adopted by Hatt.[5] When a weighed quantity of the compound was boiled in strong NaOH solution for 15 minutes and filtered, hydrated uranium (IV) oxide remained as the residue and the filtrate contained the fluoride. Uranium was determined by Jones Reductor method[6] after dissolving the residue in $6\,N.H_2SO_4$. The volumetric estimation of the fluoride present in the filtrate was carried out by the modified thorium nitrate method developed in this laboratory.[7] The analytical data recorded in Tables I, II

TABLE I

(Molecular Weight—Theoretical 341. Molecular Formula—UF_4, $1{\cdot}5\ H_2O$)

Material used for photolysis	Compound taken mg.	U_3O_8 found mg.	Molecular weight calculated	CaF_2	
				Calculated mg.	Found mg.
$UO_2(HCO_2)_2$ ..	173·0	142·8	340	79·1	79·0
	155·5	128·0	341	71·1	70·7
	147·2	121·5	340	67·3	67·0
	131·3	108·0	341	60·1	59·9
$UO_2(NO_3)_2$ ..	158·1	129·8	341·6	72·3	72·8
	134·0	110·2	341·3	61·3	61·0
	140·8	116·2	340·0	64·4	64·1
	183·0	151·0	340·3	83·7	83·5

TABLE II

Estimation of uranium by Jones Reductor and fluoride by thorium nitrate

Material used for photolysis	Compound taen mg.	Uranium		Fluoride	
		Calculated mg.	Found mg.	Calculated mg.	Found mg.
$UO_2(HCO_2)_2$ ..	160·0	111·7	111·9	35·7	34·9
	197·4	137·8	137·8	44·0	43·7
	181·2	126·5	126·1	40·4	39·8
	192·5	134·3	134·5	42·9	42·3
$UO_2(NO_3)_2$ ..	141·4	98·7	98·8	31·5	31·1
	170·5	119·0	118·8	38·0	37·9
	85·7	59·8	59·6	19·1	18·8
	81·0	56·5	56·4	18·1	18·1

and III show that the compound obtained possesses the formula UF_4, 1·5 H_2O.

TABLE III

Estimation of uranium (IV) by Hatt's method

Material used for phytolysis	Compound taken mg.	Uranium	
		Calculated mg.	Found mg.
$UO_2(HCO_2)_2$ ..	382·8	267·1	267·6
	274·5	191·6	188·2
	127·0	88·6	89·4
	147·4	102·9	101·6
$UO_2(NO_3)_2$ ..	174·4	121·7	121·0
	168·0	117·2	116·0
	111·0	77·5	77·6
	107·4	75·0	74·8

SUMMARY

A simple and convenient method for the preparation of uranium tetrafluoride utilizing solar energy has been described. The advantage of this method is that no foreign substance is used for bringing about the reduction of uranium (VI) to uranium (IV) valency state. When a solution of uranyl formate or uranyl nitrate in hydrofluoric acid and alcohol is exposed to sunlight, a bright green precipitate of uranium tetrafluoride settles down. Photolysis under similar conditions using ammonium bifluoride in place of HF gives the complex NH_4F, UF_4, H_2O.

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THE NEAR ULTRAVIOLET ABSORPTION SPECTRUM OF *PARA*-THIO-CRESOL

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Abstract

The absorption spectrum of *para*-thio-cresol consists of about ten bands in the region 2922 to 2600 Å. The bands are quite intense but broad and fail to show any structure such as is observed in the case of *para*-cresol bands. The maximum number of bands about ten is obtained by using an absorption path length of 100 cm. The (0, 0) band is chosen to be that at 34222 $cm.^{-1}$ which is the strongest band on the longer wavelength side. Vibrational frequencies in the excited state have values 353, 752 and 1114 and combinations and overtones of these are found to be present.

Introduction

Work on the absorption spectrum of *para*-thio-cresol in solution has been reported in the collection of ultraviolet spectral data of the American Petroleum Institute Research Project 44 Serial No. 437. The spectrum of *para*-thio-cresol in iso-octane solution shows one peak at about 2965 Å, the second at 2850 Å, the third at 2750 Å and the fourth at 2400 Å. Different concentrations were used to plot the graph showing the absorption against wavelength. So far no work on the vapour absorption spectrum of *para*-thio-cresol in the ultraviolet region appears to have been reported. The infra-red spectral data and the Raman spectrum data do not appear to have been reported so far for this molecule. These investigations have been undertaken. The infra-red spectrum for *para*-thio-cresol in carbon tetrachloride solution has been studied up to 900 $cm.^{-1}$ and after that the substance was dissolved in Nujol and the infra-red spectrum recorded by Perkin Elmer infra-red spectrophotometer. These results will be reported later. The ultraviolet absorption spectrum in the vapour state and the effect of changing the temperature of the absorbing column and the length of the column has been studied and reported now. Assignments have been made and discussed.

EXPERIMENTAL

Para-thio-cresol is a solid with melting point 42° C. The absorption path lengths used were from 40 cm. to 200 cm. The solid was introduced in the absorption tube and quartz windows were attached to both the open ends of the tube by sealing wax to carry out the experiment at room temperature. The tube was evacuated and sealed. For higher temperatures varying from 30–100 ° C. a tube of 40 cm. length was used. This tube was made of pyrex glass and nichrome wire was wound over the tube. The tube could be heated to various temperatures by controlling the current by means of a variac. No windows were used and ends of the tube were open to the atmosphere. There were dents provided in the tube so that the solid could be dropped through these dents from time to time. The Hydrogen lamp was used as a source for the continuum. Hilger's medium quartz and small quartz spectrographs were used to photograph the spectra with Ilford special rapid plates. Exposures of about two hours were given using the Hydrogen discharge lamp and 100 cm. path length for absorption. The bands were measured by means of a Hilger comparator, Copper or Iron lines being used as standard lines. The wavelengths of the bands were calculated by Hartmann's dispersion formula. The wavelengths of bands given in Table I represent the mean wavelengths obtained on five different plates and are expected to be correct to $\pm$ 6 cm.$^{-1}$ in the case of sharp bands and $\pm$ 10 cm.$^{-1}$ in the case of weak bands. In this case the bands being broad the readings for the band heads are taken as those corresponding to the short wavelength side.

RESULTS

Para-thio-cresol in solution phase absorbs in the region 3000 to 2600 Å and there is much stronger continuous absorption below 2600 Å. In the vapour absorption about ten bands are obtained between 2922 to 2680 Å. At room temperature, *i.e.*, 30° C. and an absorption path length of 100 cm. the bands appear between 2922 to 2680 Å. (Fig. 1 *a*). With an increase in temperature to 40° C. the bands from 2921 to 2829 Å are clearly seen while the bands below this region disappear (Fig. 1 *b*). On increasing the temperature of the absorbing column to 50° C. there is continuous absorption with the long wavelength limit at 2921 Å (Fig. 1 *c*). The strong band at 2921 Å on the long wavelength side is taken as the 0, 0 band. On taking the length of the absorption path as 100 cm. and evacuating the tube after introducing the solid and sealing it off, all the bands mentioned in Table I were recorded (Fig. 2). The data on the observed bands together with the proposed assignments are given in Table I, and the analysis discussed.

TABLE I

Near ultraviolet absorption bands of para-*thio-cresol* 2921 *to* 2680 *Å*

λ in Å	ν cm.$^{-1}$	Intensity	Separation from (0, 0), *i.e.*, 34222 cm.$^{-1}$	Assignment
2921·2	34222	*v.s.*	0	(0, 0)
2891·4	34575	*m.*	353	0+353
2858·4	34974	*m.*	752	0+752
2829·1	35336	*s.*	1114	0+1114
2801·7	35682	*m.*	1460	0+1114+353
2770·1	36089	*m.*	1867	0+1114+752
2742·6	36450	*m.s.*	2229	0+1114+1114
2716·2	36805	*w.*	2582	0+2×1114+353
2687·1	37203	*w.*	2981	0+2×1114+752

The intensities of the bands are visually estimated as mentioned here: *v.s.*, very strong; *m.s.*, medium strong; *s.*, strong; *m.*, medium and *w.*, weak.

In Table II the excited state frequencies of a number of *para*-disubstituted benzenes are compared with those obtained in the present investigation.

DISCUSSION AND ANALYSIS

The longest wavelength system in the absorption spectrum of *p*-thio-cresol lies between 2922 to 2687 Å. It consists of rather broad bands. The spectrum appears similar to that of *p*-cresol with the difference that in *p*-cresol there is more structure associated with each band. The bands of *p*-cresol, however, are spaced just like those of *p*-thio-cresol. It is also observed that the vapour spectrum of thio-phenol[5] is more diffuse than that of phenol.

Considering the *p*-thio-cresol molecule as a planar one, it belongs to C_{2v} symmetry. The transition which in the present case is the analogue of the 2550 Å transition of benzene is the allowed transition $A_1 - B_1$, and hence the strongest band on the longest wavelength side at 34222 cm.$^{-1}$ is taken as

the 0–0 band. The temperature effect corroborates the choice of the 0–0 band.

TABLE II

Table showing the comparison of the excited state frequencies in p-*disubstituted benzenes*

p-dichloro-benzene[9]	*p*-dichloro-benzene[8]	*p*-fluoro-chloro-benzene[3]	*p*-Xylene[2]	*p*-cresol[6,7]	*p*-cresol[4]	*p*-chloro-toluene[10]	*p*-fluoro-toluene[1]	*p*-thio-cresol Present Expt.
35,750	35,743	36,276	36,733	35,337	35,337	36,299	36,876	34,222 (0, 0) band
331	531	343	367	210	217	796	185	353
528	728	563	552	419	420	1044	398	752
725	..	618	775	808	806	1068	584	1114
1051	..	794	802	1187	1194	1189	794	..
1061	..	1063	1185	1268	1273	..	843	..
1461	..	1233	..	..	..	..	1014	..
..	..	..	..	..	..	..	1194	..
..	..	..	..	..	..	..	1229	..
v.v.	*v.v.*	..	*v.v.*	..	*v.v.*	*v.v.*	*v.v.*	..
26	25	..	12	..	38	26	40	..
86	84	..	27	..	2×38	57	96	..
..	..	..	39	..	161	100	..	..
..	..	..	53	..	231	..	..	..
..	..	..	72	..	..	..	..	..
..	..	..	92	..	..	..	..	..

The analysis proposed in Table I indicates that all the bands can be accounted for on the basis of three frequencies in the excited state namely 353, 752 and 1114 cm.$^{-1}$ It may be observed that the band at 35336 cm.$^{-1}$

is a strong band next in intensity to the 0–0 band. It has been assigned as due to the fundamental 1114 and not as 353 + 752 = 1105 on intensity considerations. The combination of these two frequencies is not expected to be stronger than those involving only one quantum.

In Table II, the data regarding the excited state frequencies involved in various disubstituted benzenes has been given. On the basis of this comparison it is observed that most of these molecules have a frequency in the range 725 to 825. The bands involving such a frequency in the excited state are usually strong in the spectra. These frequencies are assigned to be corresponding to 992, the ring breathing vibration of benzene in the excited state. In the present case 752 has been taken to be the corresponding frequency. It occurs in combination with other frequencies. The frequency 1114 cm.$^{-1}$ is chosen in the present case as another fundamental in the excited state. In Table III it is shown that a frequency of the order of 1180 is always present in the spectra of substituted benzenes which has at least one methyl group as substituent. In those cases where the corresponding emission spectra have been investigated the corresponding ground state frequency is found to be of the order of 1210. It is also observed by comparing Tables II and III that a frequency of 1180 or so is absent in those cases where

TABLE III

Ground and excited state frequencies of disubstituted benzenes having CH_3 as one of the substituents

Molecule	Ground state or Raman or infrat-red frequency cm.$^{-1}$	Excited state frequency cm.$^{-1}$	Author
p-Fluorotoluene ..	1213	1194	W. T. Cave and H. W. Thompson[1]
p-Chlorotoluene ..	1208	1189	G. Viswanath[9]
p-Cresol ..	1213 or 1251	1187	H. Sponer[6]
p-Xylene ..	1208	1185	C. D. Cooper and M. L. N. Sastri[2]
p-Thio-cresol ..	1211 (infra-red)	1114	Present work

there is no methyl group as one of the substituents, *i.e.*, in *p*-dichloro-benzene and *p*-fluoro-chloro-benzene. It is thus apparent, that the frequency of the order of 1180 cm.$^{-1}$ corresponds to C—CH_3 stretching vibration, the value in the ground state being of the order of 1210 cm.$^{-1}$ The assignment of the two frequencies namely one between 725 to 825 and the other near 1150 to 1200 has been a subject of controversy. In the present case the frequency 1114 seems to be rather low as compared to that in *p*-cresol, *p*-xylene and other molecules as shown in Table II. A preliminary study of the infra-red spectrum, however, shows that the value in the ground state is probably of the same order as in *p*-cresol. This assignment, however, is tentative. If this is confirmed by further work on the three thio-cresols it will show that the ground state C—CH_3 valence frequency remains unchanged in the SH-substituted compounds, while the excited state gets heavily reduced. This would have an important bearing on the electronic structure of the excited state.

It will also be observed that 0–0 band of *p*-thio-cresol is shifted by 1115 cm.$^{-1}$ to longer wavelength. Table II shows that it is situated at the longest wavelength as compared to the 0–0 bands of other disubstituted benzenes.

SUMMARY

The near ultraviolet absorption spectrum of the *p*-thio-cresol molecule which has been studied shows about ten broad bands in the 2800 Å region. Fundamental frequencies in the excited state have values 353, 752 and 1114 cm.$^{-1}$ and a few combinations and overtones of these are found to be present. The shift of the 0–0 band in *p*-thio-cresol is well marked when SH is substituted in place of OH in *p*-cresol. The frequency near 1180 is assigned as due to C—CH_3 vibration. The excited state frequencies in case of various disubstituted benzenes have been compared and discussed.

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FIG. 1. Absorption bands of *p*-Thio-cresol at different temperatures.

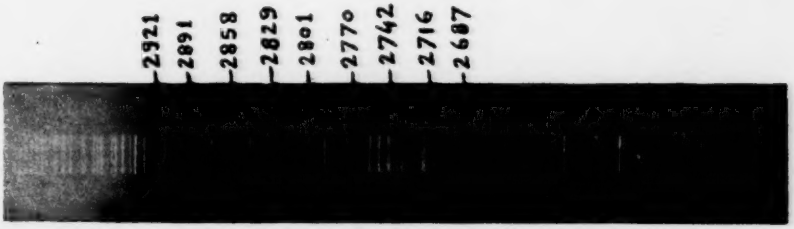

FIG. 2. Absorption spectrum of *p*-Thio-cresol.

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STUDIES ON COLLAGEN

IV. Deposition of Inorganic Crystallites on Collagen

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1. Introduction

This paper deals with a study of the deposition of inorganic crystallites of collagen fibres by X-ray diffraction methods. It was observed in this laboratory (Ambady, 1959) that salts like sodium carbonate, sodium sulphate and sodium fluoride could be crystallized inside collagen fibres by treating the fibres with the respective salt solutions and in the case of sodium carbonate by mere treatment with sodium hydroxide solution. In the present study, various salts, soluble and insoluble, were tried and the results obtained are presented here. A study of the deposition of calcium phosphate on collagen was also made with a view to understand the phenomena of bone formation. This has been described separately in another paper (Santhanam, 1959).

2. Experimental Methods

Collagen fibres were treated with 10 per cent. solutions of the inorganic salts in the case of soluble compounds. The fibres were kept in these solutions for a period of 24 hours, and were then taken out and dried. The X-ray patterns were recorded in a 3 cm. cylindrical camera. In the case of insoluble substances a 10 per cent. suspension in water was employed.

3. Results

Figures 1, 2 and 3 are the patterns of specimens treated with solutions of silver nitrate, lead acetate and barium chloride. Figure 4 is the pattern of a specimen treated with a suspension of calcium carbonate. The same pattern is obtained by keeping the fibres for a long time in Ringer solution. Figure 5 is the pattern of a specimen treated with a suspension of calcium fluoride. Figure 6 is the pattern of a specimen treated with a suspension of bismuth oxychloride (bismuth chloride in water). Some other solutions like magnesium sulphate and strontium nitrate were also tried but it was observed that these substances did not crystallize inside the fibres.

The substance that crystallizes inside the fibre by treatment with silver nitrate solution has been identified as silver oxide. The spacings and intensities of the observed pattern agree completely with the data reported for silver oxide in A.S.T.M. index card No. 3·0796. In the case of treatment with lead acetate solution the substance has been identified as basic lead carbonate as the spacings and intensities of the pattern agree with those given for white lead in A.S.T.M. card No. 1·0687. In the case of barium chloride, calcium carbonate, and bismuth oxychloride the respective salts themselves crystallized inside the fibres and the spacings and intensities of the patterns agree with those given for these substances in A.S.T.M. index cards Nos. 1·0342, 5·0586 and 1·0600 respectively.

4. Conclusions

In the case of silver nitrate it was observed that the substance which crystallized inside the fibres was silver oxide and not silver nitrate. It was observed that the same silver oxide was deposited when the fibres were treated with other silver salt solutions like silver sulphate. The formation of silver oxide suggests that there may be the initial formation of unstable silver hydroxide which subsequently decomposes into silver oxide. As is well known, the silver nitrate solution itself is perfectly stable and no precipitate is observed in the absence of collagen. So also no precipitate is deposited in the vessel itself but only on the collagen fibres.

In the case of lead acetate the substance that crystallized inside the fibres was found to be basic lead carbonate. Here also it was observed that the same white lead crystallized even if other salt solutions like lead nitrate were employed. As with silver nitrate, there is a deposit only on collagen and not if there is no collagen present. However, exposure to atmosphere was found to be essential for crystallisation to occur in the case of lead salts and no crystallisation occurred inside the fibres if the fibres were treated with lead acetate solution in closed bottles. The deposition of basic lead carbonate and not the salt used in the solution suggests that lead hydroxide is first formed which subsequently interacts with the atmospheric carbon dioxide, to form the basic salt. It is interesting to observe that with both silver and lead, there is the formation of the metallic hydroxide in the presence of collagen.

The amino acid composition of collagen shows an excess of basic groups. Thus free carboxylic groups form 0·77 mg. equiv. whereas the content of basic groups is 0·87 mg. equiv. leading to a surplus of 0·1 mg. equiv. of basic groups (Bowes and Kenten, 1948). This is responsible for the location of the iso-electric point of native collagen in the slightly alkaline range of pH 7–8 (Kramer and Dexter, 1927; Briefer, 1929). Collagen is an ampho-

teric ionic structure and the charged centres of the protein zwitterions exist in electrostatic balance with each other. This may be treated as a salt of a weak acid and a weak base so that both the cations and anions undergo hydrolysis according to the equation

$$B + A + H_2O \rightarrow BOH + HA$$

Whether the solution is basic or acidic will be determined by the relative strength of acidic and basic groups. The presence of excess of basic groups in collagen makes the solvent medium basic and this leads to the formation of silver hydroxide and lead hydroxide in the presence of silver nitrate and lead acetate. The two metals are known to form their hydroxides readily in the presence of a slightly alkaline medium and the hydroxides could subsequently interact with atmospheric carbon dioxide.

In the case of soluble salts, the salts could be removed by washing the fibres with water, while in the case of insoluble salts they are not removed by washing.

The deposition of lead in the form of basic lead carbonate is of interest in the understanding of the physiological behaviour of lead salts. All soluble lead salts are poisonous and lead poisoning is usually cumulative. The biochemical behaviour of lead has been the subject of many investigations (Sobel *et al.*, 1940) and there is general agreement that lead and phosphorus form a system for lead deposition analogous to the well established system for calcification. The present finding that, in the presence of carbonate ions all soluble lead salts are deposited in the form of white lead, may be of interest in connection with the biochemical aspects of lead-poisoning.

The reason why certain soluble salts are deposited on collagen while others are not, has been attributed to the approximate equality of a multiple of a repeat spacing to the collagen fibre axis repeat of 28·6 A in one case and the absence of such a relationship in the other (Ambady, 1959). It may be mentioned that this has been confirmed in the case of salts like barium chloride, silver oxide, calcium carbonate, calcium fluoride, magnesium sulphate and strontium nitrate studied here. The relevant lattice constants of the various compounds studied here are given in Table I and it will be seen that the integral multiple of one of the constants is close to 28·6 A in the case of calcium carbonate, silver oxide and barium chloride dihydrate. In the case of calcium fluoride the spacing of (111) planes is $5 \cdot 46/\sqrt{3} = 3 \cdot 15$ A and nine times this value is 28·4 A. All these compouds are seen to crystallize readily in the collagen. On the other hand none of the multiples of the lattice constants of magnesium sulphate and strontium nitrate are

close to the fibre axis repeat spacing of collagen and, as already mentioned, these compounds do not crystallize on collagen.

TABLE I

Comparison of a multiple of the lattice constant with the fibre axis repeat of collagen for the compounds studied

Compound	System	Unit Cell	Multiple of a lattice constant
1. Calcium carbonate ..	Hexagonal	a=4·12 A c=8·556 A	$7a$=28·84 A
2. Calcium fluoride ..	Cubic F.C.	a=5·4626 A	$9d_{111}$=28·4 A
3. Silver oxide ..	Cubic	a=4·72 A	$6a$=28·32 A
4. Magnesium sulphate ..	Orthorhombic	a=11·91 A b=12·02 A c=6·877 A	..
5. Strontium nitrate ..	Cubic	a=7·779 A	..
6. Barium chloride dihydrate	Monoclinic	a=6·74 A b=10·86 A c=7·14 A β=91·5	$4c$ = 28·56 A

5. SUMMARY

The paper deals with a study of the deposition of inorganic crystallites on collagen fibres. It is shown that some soluble and insoluble salts could be deposited inside the fibres by keeping them in neutral solutions or suspensions in water in the case of insoluble salts. Thus barium chloride dihydrate could be deposited by keeping the fibres in a neutral solution of barium chloride, while calcium carbonate and calcium fluoride could be deposited by keeping the fibres in suspensions of these salts. In the case of silver and lead salts, the substances crystallizing inside the fibres have been identified as silver oxide and basic lead carbonate respectively.

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DESCRIPTION OF PLATE IX

All photographs are X-ray diffraction patterns taken with CuKα radiation in a cylindrical camera (R = 3 cm.).

FIG. 1. Collagen deposited with silver oxide.

FIG. 2. Collagen deposited with white lead.

FIG. 3. Collagen deposited with barium chloride dihydrate.

FIG. 4. Collagen deposited with calcium carbonate.

FIG. 5. Collagen deposited with calcium fluoride.

FIG. 6. Collagen deposited with bismuth oxychloride.

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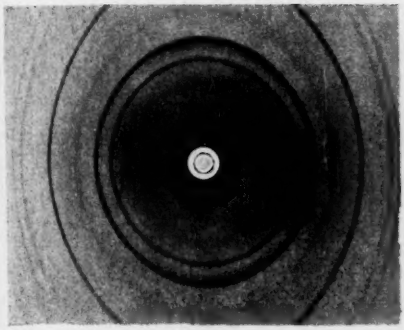

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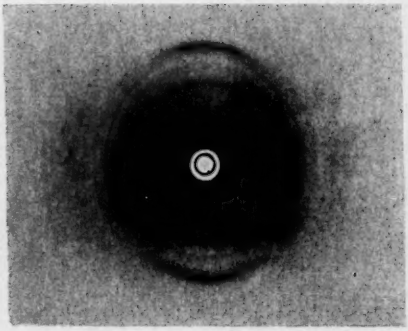

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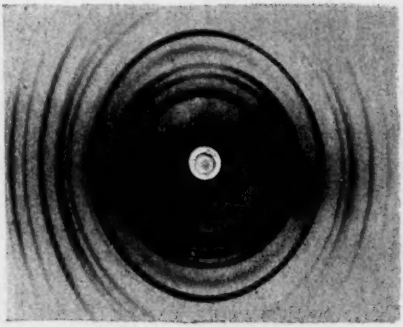

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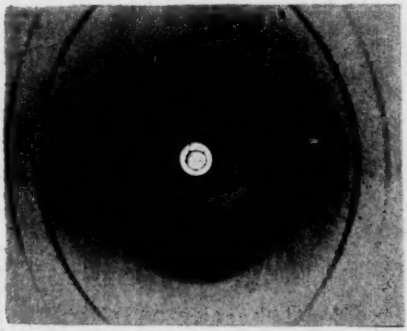

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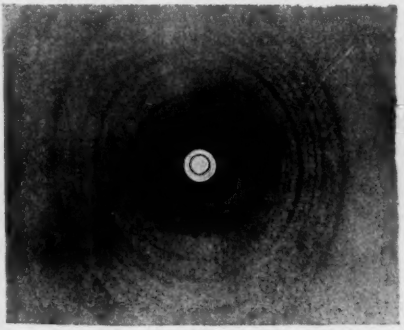

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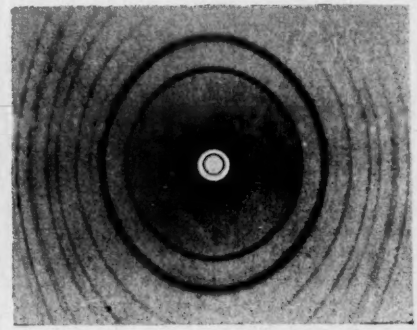

FIGS. 1–6

STUDIES ON COLLAGEN

V. Modification of Collagen by Neutral Salt Solutions

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(Communicated by Prof. G. N. Ramachandran, F.A.SC.)

Introduction

The basic features of the backbone of the collagen structure are now fairly well established (Ramachandran, 1956). The structure consists of three helical polypeptide chains each of which has ten residues in three turns of a left-handed helix. The three chains are further wound into a superhelix in the form of intertwined coiled-coils. These are linked together by means of interchain hydrogen bonds between the imino hydrogen and carbonyl oxygen atoms. This may be called the protofibril of collagen. The protofibrils are linked together mainly through the hydroxyproline side-chains, the OH group of the side-chain being bound to a carbonyl oxygen of a neighbouring protofibril by a hydrogen bond. However, the finer details of the structure are not yet clear.

With a view to gain a better understanding of the structure of collagen and the nature of bonds stabilising its structure, work was begun on the modifications induced in the collagen structure by chemical treatment. Studies were first made with neutral salt solutions of the lyotropic type and their influence on the collagen structure was followed. Although the effect of salt solutions on collagen has been fairly well investigated from a chemical viewpoint, this appears to be the first time that they have been studied by X-ray diffraction methods. So also the lyotropic nature of nickel nitrate solution seems to have been recognised for the first time during this study. In fact, the effect of this particular salt was studied in great detail. These studies have resulted in the postulation of a structure for elastin similar to that of collagen (Ramachandran and Santhanam, 1957) and have led us to make some suggestions of the biosynthesis of collagen and elastin which are discussed in the last section of this paper.

Among the chemical studies made earlier, may be mentioned in particular that of Thomas and Kelly (1927) who found the results to be typical of the Hofmeister ion series and considered the destructive effect as one of hydrolysis. Gustavson (1956 *a*) on the other hand considered that the

neutral salt solution does not bring about any hydrolysis and suggested that they only produce a weakening and breakage of intermolecular forces between the protein subunits (hydrogen bonds). Our studies show that hydrolysis does not occur during treatment with neutral salt solutions, for the changes which are induced in collagen are reversed, and the original structure is regained when the salt is washed off. In fact the value of the work arises from this, namely that the modifications induced are not chemical but purely structural.

2. Experimental Study of Shrinkage

Collagen fibrils from cattle Achilles tendon, horse Achilles tendon, kangaroo tail tendon, and rat tail tendon were used for the investigation. Fibres of the same length and thickness were used. The time taken for complete shrinkage in various lyotropic agents like nickel nitrate, calcium chloride, potassium iodide and lithium chloride were determined. The times of shrinkage in the various lyotropic agents of varying concentration are given in Table I.

It will be noticed that the time of shrinkage varies very much from specimen to specimen even in the same solutions. These differences are to be attributed to the variations in the texture of the fibres rather than to any variations in chemical composition. This view receives support from the observed fact that reduction of thickness of the fibres decreased the time of shrinkage even with the same specimen. A similar observation namely that rat tail tendon and icthyocoll dissolve easily in dilute acids while skin collagen only swells and is not dissolved in dilute acid has been attributed by Gustavson (1956 *b*) to the differing weave structure of the two specimens.

The shrinkage does not appear to be due to the effect of any single ion of the salt, but is probably characteristic of the compound as such. This is shown by a study of shrinkage in various salt solutions containing the respective cations and anions. Thus it was observed that while shrinkage took place readily in nickel nitrate solution, no shrinkage was observable in solutions of potassium nitrate, sodium nitrate, ammonium nitrate and barium nitrate of the same concentration. So also, shrinkage took place in nickel chloride solution as in nickel nitrate but in the case of nickel sulphate (saturated solution), there was no visible shrinkage. It appears that the effect is due to the neutral salt as a whole and does not depend on any particular ion. As may be seen from Table I, the time of shrinkage increases with decreasing concentration and after a limiting concentration, shrinkage does not occur at all. This is understandable, if we suppose that the shrinkage temperature is lowered by a definite amount for a particular

concentration. If the chemical reagent weakens the linkages (like hydrogen bonds) which stabilise the structure, then shrinkage temperature would obviously be lowered because the thermal energy required for the reaction would be reduced, since part of the activation energy is supplied by the chemical effect. So also, at any particular temperature the rate of reaction would be faster, the higher the concentration of the chemical.

TABLE I

Shrinkage of collagen in neutral salt solutions. The time for shrinkage is given in seconds

	Percentage concentration (W/V)					
	100	80	67	50	40	33
			1. *Nickel Nitrate*			
C.A.T. ..	100	130	150	180	200	..
H.A.T. ..	110	..	150	..	200	..
R.T.T. ..	10	20	30	50	70	80
K.T.T. ..	8	12	15	40	50	60
			2. *Calcium Chloride*			
C.A.T. ..	150	..	200	..	320	..
H.A.T. ..	160	..	270	320	400	..
R.T.T. ..	3	..	10	20	35	50
K.T.T. ..	3	..	8	20	30	45
			3. *Potassium Iodide*			
C.A.T. ..	30	..	40	40	50	..
H.A.T. ..	40	..	50	60	70	..
R.T.T. ..	4	..	6	7	7·5	8
K.T.T. ..	1	..	1·5	..	2	..

3. X-RAY STUDY OF SHRINKAGE

The changes in the ultimate structure which occur in the process of chemical shrinkage have been followed by X-ray diffraction studies and the results are presented below. The observed time of shrinkage was divided into convenient intervals and fibres of C.A.T. of the same size were treated with salt solutions of concentration 100 per cent. (W/V) for different intervals of time. The reactions were arrested at the desired stage by taking the fibres out and blotting them with bits of filter-paper, thus removing the reagent. It was observed that the treated specimens could be stored for any length of time and they underwent no further change, which was shown by the fact that the X-ray patterns were always the same.

4. EXPERIMENTAL RESULTS

(*A*) *Nickel Nitrate.*—The various X-ray diffraction patterns obtained by treatment with nickel nitrate solution are given in Figs. 1–6. Figure 1 is the pattern of the specimen treated with the salt solution for 20 seconds. The pattern does not reveal any change from the normal collagen pattern. The pattern of the specimen treated with nickel nitrate solution for 40 seconds is given in Fig. 2. In this pattern, the equatorial spot spacing increases to a value of 12·5 A. Besides the 2·86 A meridional arcs, there are two other meridional arcs with spacings at 3·9 A and 4·8 A and a fourth meridional reflection at 9·33 A is also seen.

Figure 3 is the pattern of the specimen treated for 60 seconds. Here also, the double arcs with spacings corresponding to 3·9 A and 4·8 A are clearly visible. The 9·33 A meridional reflection is also seen. There is a contraction in the width of the equatorial spots and their spacing increases to a value of 13·8 A. The spacing of the third layer spots comes down to 6·73 A from the usual value of 7·8 A. The ξ value of these spots increases from the normal value of 0·12 to 0·17. This has been accurately measured by recording a pattern of the fibre in the stretched condition.

Figure 4 is the pattern of the specimen treated for 65 seconds. Here the 2·86 A arcs are recorded very faintly and the equatorial spots (10–12 A) also are not clearly recognisable. The two meridional arcs at 3·9 A and 4·8 A seen in Figs. 2 and 3 have coalesced into a diffuse ring with a maximum at 4·4 A. The pattern shows a diffuse halo around the central spot in addition to the diffuse ring at 4·4 A.

Figure 5 is the pattern of the fibre treated for 80 seconds. In this pattern the halo surrounding the central spot seen in Fig. 4 disappears and the pattern shows only a diffuse ring at 4·4 A, with very little intensity inside. This represents the ultimate stage in the degradation of the collagen structure,

since further treatment does not produce any more change. The pattern is similar to that of gelatin sol (Katz *et al.*, 1933) and of drastically shrunk collagen (Astbury, 1940). All these patterns show a diffuse ring at 2·2 A, if the patterns are recorded in a cylindrical camera. On washing the fibre with distilled water, the normal collagen pattern is regained (Fig. 6).

(*B*) *Calcium Chloride.*—Figure 7 represents the pattern of the specimen treated with calcium chloride solution for 40 seconds. The pattern does not show any change from the normal collagen pattern. Figure 8 is the pattern of the specimen treated for 120 seconds. Here the 2·86 A arcs and the 10–12 A equatorial spots have disappeared and the pattern shows only a central halo with a maximum at about 9 A, and another diffuse ring at 4·4 A. This pattern is seen to exhibit a remarkable similarity to that of elastin given in Fig. 9. Figure 10 is the pattern of the specimen treated for 160 seconds. It shows an intensification of the 9 A ring and the diffuse ring at 4·4 A is also seen in the pattern. On washing the fibre with distilled water, the normal collagen pattern is regained. The figure is not reproduced.

(*C*) *Potassium Iodide.*—The pattern of the specimen treated for 10 seconds is given in Fig. 11. This pattern does not show any difference from ordinary collagen. Figure 12 is the pattern of the specimen treated for 20 seconds. A general diffuseness is observable and there is a thinning of the 10–12 A equatorial spots. The 2·86 A arcs are also faint. The pattern of the specimen treated for 30 seconds is given in Fig. 13. It shows a ring at 9 A and some powder lines due to potassium iodide. On washing, here also the normal pattern is regained (not reproduced).

(*D*) *Lithium Chloride.*—The pattern of the specimen treated with lithium chloride solution for 30 seconds is given in Fig. 14. The 2·86 A arcs are faint and there is a thinning of the 10–12 A equatorial spots. A diffuse ring at about 3–4 A is visible and the pattern shows clear signs of disorientation. Figure 15 is the pattern of the specimen treated for 60 seconds. It shows a central halo with a maximum at about 9 A and another diffuse ring at 3·2 A. Figure 16 is the pattern of the specimen treated for 90 seconds. In this pattern the maximum at 9 A seen in Fig. 15 is not observable and the pattern shows a diffuse halo around the central spot extending to about 9 A and a diffuse ring at 3·2 A. The patterns in Figs. 15 and 16 are very similar to the pattern of wet gelatin given by Hermann *et al.* (1930). On washing the fibres with distilled water, the normal pattern is regained.

5. Interpretation of the Results

The previous paper by the authors (Ramachandran and Santhanam, 1957) on the structure of elastin dealt with only certain aspects of the pheno-

mena reported here namely those related to elastin and contained only a short statement of the overall picture. From the results recorded here, a general similarity is noticeable in the effects of the different lyotropic agents, notably in the initial stages of degradation, although in the later stages the transformations are not identical. In the case of nickel nitrate treatment, a complete similarity between the shrinkage brought about by this reagent and thermal shrinkage is observable, as shown by the X-ray patterns. In the case of thermal shrinkage also several patterns corresponding to intermediate stages of disruption of collagen, as in nickel nitrate treatment, were obtained by partial recovery of the normal ordered structure from the thermally shrunk state. But it has not been possible to stop the thermal shrinkage at controlled stages, as in the case of lyotropic treatment.

It is interesting to observe that, at an appropriate stage in nickel nitrate and calcium chloride treatments, collagen exhibits an X-ray pattern similar to elastin. In nickel nitrate, this happens between 60 and 80 seconds (Figs. 3 and 4) and by suitable adjustment of the time of treatment, it has been possible to record a pattern very closely similar to that of elastin. In calcium chloride, after treatment for 120 seconds, the X-ray pattern almost exactly resembles that of elastin and in fact the similarity is so great that one can easily be mistaken for the other. By drastic treatment with nickel nitrate, an X-ray pattern is obtained which is identical with that of gelatin sol (Katz *et al.*, 1933) and drastically shrunk collagen (Astbury, 1940). The fact that, in all these treatments, the normal collagen pattern is regained by washing with water indicates that no alteration takes place in the polypeptide chains as such during the process and that the changes are only due to the breaking of secondary valence bonds between the chains.

At the stage when the X-ray pattern of the treated specimens resembles that of elastin, both the 2·86 A arcs and 10–12 A spots disappear and the pattern shows only two diffuse rings at 4·4 A and 2·2 A besides a central halo. These changes could be interpreted as being due to the ordered structure of normal collagen being broken down by chemical treatment. The 2·86 A meridional arc in the collagen pattern is due to the order arising from the helical arrangement of residues in the individual triple chain protofibril, while the 10–12 A spot is due to the order arising from the arrangement of the triple chain protofibrils in a lattice. These two types of order are interrelated and the occurrence of the sharp ring at 2·86 A is also to be attributed to the cross-links between the protofibrils, which would lead to the repeat spacing being the same in all protofibrils, thus producing a high regularity,

It is believed that hydroxyproline serves as the main cross-linking agent in collagen, the hydroxyl groups of the side-chain being bound to a carbonyl oxygen of a neighbouring protofibril by a hydrogen bond. As the five-membered ring in hydroxyproline is a highly rigid group, this linkage is likely to keep the distance between the neighbouring protofibrils uniformly the same and also make the repeat spacing along the fibre axis to be the same in all protofibrils. When these cross-links are broken, as in the lyotropic treatments, both types of order are lost, resulting in the folding and twisting of the single protofibrils leading to complete disorientation. But the fact that the broad rings at 4·4 A and 2·2 A, which are observable in gelatin, are present in these patterns, suggests that the triple helical structure is maintained in each protofibril, although the lattice structure and regularity are lost.

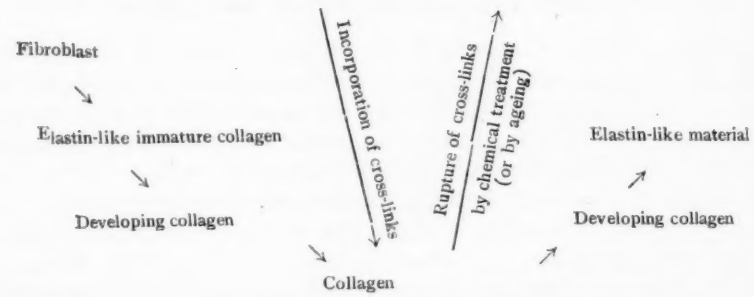
In the final stages of nickel nitrate treatment, the pattern resembles that of hot gelatin and here it appears likely that the individual chains in the triple chain separate. These findings are also in agreement with the data on optical activity and infra-red absorption. The specific rotation of solutions of collagen and cold gelatin is about $-300°$ while it changes to $-110°$ when the solution is heated to 30° C. (Cohen, 1955). The latter value is the same as the mean specific rotation of the constituent amino-acids and so it is probable that no specific helical structure occurs in the degraded state above 30° C. The increased specific rotation at a lower temperature indicates the occurrence of a characteristic collagen helix, which may be identified with the triple helical structure. Similarly the NH stretching frequency has a value of 3,330 $cm.^{-1}$ in normal collagen and it comes down to a value of 3,310 $cm.^{-1}$ in hot gelatin (Robinson, 1953). The lower value is also found in the case of developing tendon of a rat three days old.

6. Biosynthesis of Collagen and Elastin

In Figs. 2 and 3 a significant doubling at spacings corresponding to 3·0 A and 4·8 A is observable and these patterns show a remarkable similarity to the pattern of developing collagen (rat tail tendon 9 days old) given by Jackson *et al.* (1953). The next stage in the lyotropic transformation results in the production of an elastin-like structure. This pattern shows a close similarity to the pattern of immature collagen (rat tail tendon 3 days old) given by the same authors. Thus it has been possible to deform natural collagen into structures resembling immature collagen and developing collagen. The specimens chemically treated this way should be regarded as intermediate stages in the degradation of collagen structure, since they could be kept for months without change. Thus it has been possible to obtain in the laboratory the various stages occurring in the natural *in vivo*

formation of collagen, though of course in the reverse order. The results seem to indicate that the highly ordered collagen system develops from an elastin-like structure which occurs in immature collagen and the developing collagen marks an intermediate stage between immature collagen and adult collagen. The elastin system is characterised by the absence of cross-links between the protofibrils and the development of tendon appears to be due to the incorporation of more and more cross-links between the protofibrils resulting in the production of the highly ordered collagen system. Thus it appears that the elastin-like immature collagen is a biological precursor of collagen.

These results form a useful basis for discussing connective tissue changes which occur *in vivo*. Hall *et al.* (1955) have suggested two pathways for the biosynthesis of collagen and elastin. In the first it is assumed that both collagen and elastin are synthesised simultaneously by the selection of different groupings of polypeptide and polysaccharide fragments. The other pathway assumes the preformation of collagen and subsequent degradation leading to the elastin system. The studies reported here point to a third possibility not considered by the above authors namely that elastin (elastin-like immature collagen)* is a biological precursor of collagen and that the organised structure of collagen is formed from an elastin-like structure of immature collagen by the incorporation of cross-links between the protofibrils. The breakdown of collagen to an elastin-like structure is brought about by chemical treatment and a similar breakdown may also take place in senile conditions. This suggestion is in harmony with the finding of Banga (1953) that collagen thermally shrunk or treated with phosphotungstic acid is attacked by elastase. The above picture may be represented by the following scheme:



* The author wants to make it clear that he is not suggesting that elastin as such is a precursor of collagen. The suggestion is that in the early stage, the protofibrils in the structures are not cross-linked and thereby well-oriented as in mature collagen, although the triple helical structure is present (as in elastin).

The above mechanism does not require the postulation of separate elastoblastic cells specialized in function to produce elastin fibres separate from those which produce collagen fibres but only one type of fibroblast which produce elastin-like fibres, which later develop into collagen fibres. This also explains the very close association of the two types of fibres in connective tissue and the occurrence of intermediate types.

The side-groups of hydroxyproline serve for cross-linking the adjacent protofibrils and collagen is the only protein that contains this amino-acid in appreciable quantity. It will be of interest to examine how the hydroxyproline required for the synthesis of collagen is obtained. There are two possibilities for this. One is that hydroxyproline as such is incorporated by the tissue from outside it and the second is that proline is first taken in and this is later hydroxylated inside the connective tissue. Two recent studies point to the latter possibility. Smith and Jackson (1956) have observed that collagen-forming tissue is able to hydroxylate L-proline into hydroxyproline by isotopic tracer studies. Stetten and Schoenheimer (1944) have shown that there is a conversion of L-proline into hydroxyproline when L-proline is administered into intact rats and in a recent paper (Smith and Jackson, 1957) the suggestion made here that proline is first incorporated in the protein chain, which is later converted into hydroxyproline has been confirmed.

7. Addendum

After this paper had been prepared, two recent papers have come to the author's notice which lend strong support to the suggestions made here regarding immature collagen and the biosynthesis of collagen. F. Hartmann and co-workers (1958) in an X-ray study of the structure and function of tissue have reported that the collagenous fibres of newborn rat did not show fibre diagrams. They have also observed that the collagen structure is lost by swelling with dilute acids and alkalies and that the structure is recovered if the acid or alkali is washed out, very similar to the observations reported in this paper. In a study of the development of collagen in frog embryo, Mac. V. Edds (1958) has reported that hydroxyproline first appears in *Rana pipiens* in his stage 21–22 (after 172 hours) and increases linearly thereafter. This stage would correspond to the stage of developing collagen, the stage at which, according to the ideas developed in this paper, the cross-linking process has just commenced. A fuller and detailed study of the development of collagen from other standpoints may be expected to throw further light on the role of hydroxyproline in the biosynthesis of collagen.

8. SUMMARY

The paper deals with an X-ray study of the influence on the structure of collagen of certain neutral salt solutions, which produce shrinkage at room temperature. The transformations occurring in the collagen structure were followed by recording the X-ray diffraction patterns of the fibres treated in the solutions for varying intervals of time. The X-ray patterns showed a remarkable similarity to those of immature collagen and developing collagen in the treatment with nickel nitrate solution and to that of elastin in the treatment with calcium chloride solution. It is inferred from these, that the highly ordered collagen fibres in biological systems develop from an elastin-like structure, which occurs in immature collagen and that the developing collagen marks an intermediate stage. The changes induced in the collagen structure by chemical treatment are reversible and the original structure is regained when the salt is washed off, indicating that the transformations are purely structural and not chemical. On the basis of these studies some new suggestions are made on the biosynthesis of collagen and elastin.

9. ACKNOWLEDGEMENT

The author wishes to express his grateful thanks to Professor G. N. Ramachandran for valuable guidance and interest he took in the course of this study.

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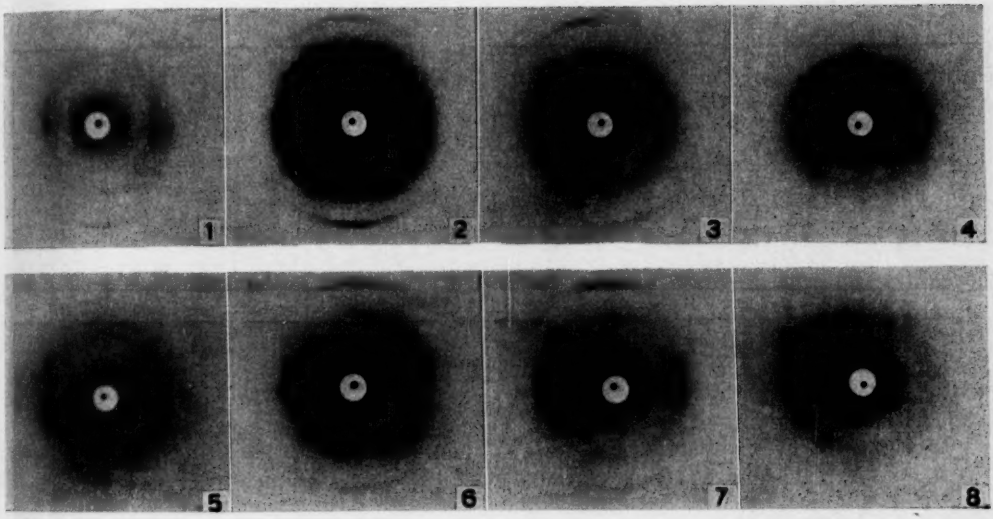
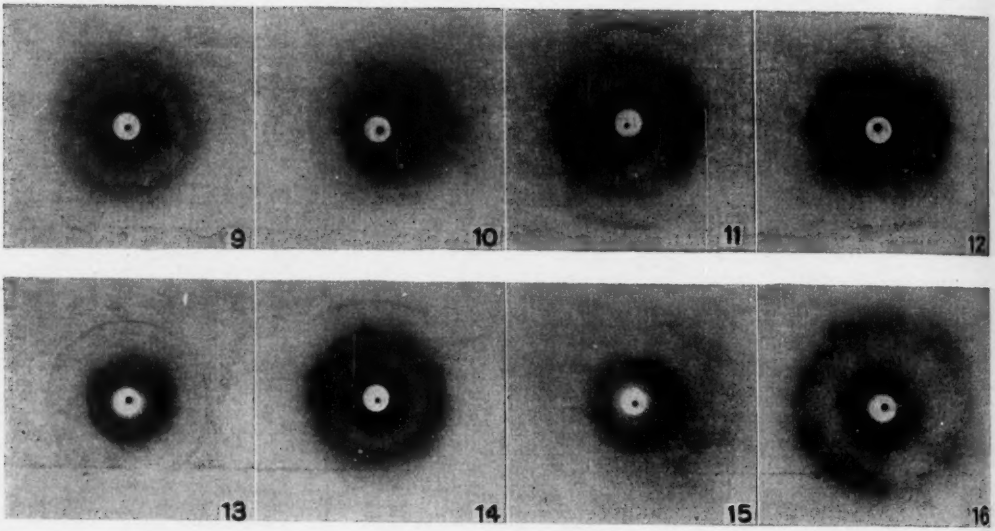


FIGS. 1–8



FIGS 9–16

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Description of Plates

All photographs are X-ray diffraction patterns taken with CuKα radiation in a flat plate camera ($d = 3$ cm.). The salt solutions employed are 100 per cent. (W/V).

Plate X

(A) *Nickel Nitrate Solution*

Fig. 1. Collagen treated for 20 seconds.
Fig. 2. Collagen treated for 40 seconds.
Fig. 3. Collagen treated for 60 seconds.
Fig. 4. Collagen treated for 65 seconds.
Fig. 5. Collagen treated for 80 seconds.
Fig. 6. Specimen 5 washed with distilled water and air-dried.

(B) *Calcium Chloride Solution*

Fig. 7. Collagen treated for 40 seconds.
Fig. 8. Collagen treated for 120 seconds.

Plate XI

Fig. 9. X-Ray pattern of elastin.
Fig. 10. Collagen treated for 160 seconds.

(C) *Potassium Iodide Solution*

Fig. 11. Collagen treated for 10 seconds.
Fig. 12. Collagen treated for 20 seconds.
Fig. 13. Collagen treated for 30 seconds.

(D) *Lithium Chloride Solution*

Fig. 14. Collagen treated for 30 seconds.
Fig. 15. Collagen treated for 60 seconds.
Fig. 16. Collagen treated for 90 seconds.

STUDIES ON THE COMPLEX FORMATION OF BORON WITH AROMATIC HYDROXY COMPOUNDS

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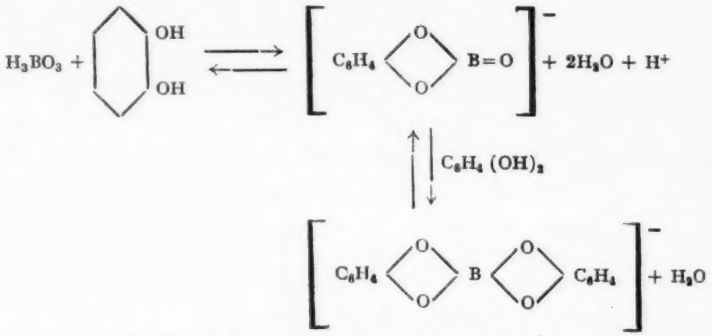
Received September 5, 1958

(Communicated by Dr. H. J. Bhabha, F.R.S., F.A.SC.)

Introduction

The reactivity of boron with organic compounds containing the OH group is well known. Mannitol and other alcohols have been widely used for the titration of boric acid. The presence of alcohol in a solution containing boric acid results in complex formation of the borate ion with the former and hence the liberation of an equivalent amount of hydrogen ions. It, therefore, seemed interesting to study complex formation equilibria in aqueous solution with some aromatic hydroxy compounds which are well known for their complex-forming characteristics.

It is known that boric acid forms complexes with *o*-hydroxy compounds such as catechol, by the following equilibria:



Knowing the dissociation constant of catechol the formation constant of mono- and dicompounds can be easily determined following Bjerrum's[1] method of stepwise addition of ligands to boron. If we represent the concentration of boron by M and the ligand concentration by A, then the reactions may be represented as:

$$M + A \underset{\rightleftharpoons}{k_1} MA \quad (1)$$

$$MA + A \underset{\rightleftharpoons}{k_2} MA_2 \quad (2)$$

Following Bjerrum's[1] method, pH titration and subsequent calculation can give the values of $\bar{n}$, the average number of ligands complexly bound to each boron atom under any set conditions, and also the value of (A), the actual concentration of the ligand that is not protonated or complexly bound. The method of calculation has also been described by Maley and Mellor.[2] When $\bar{n} = 0{\cdot}5$, (A) corresponds to the stability of the complex.

Experimental

Bjerrum's[1] method for the determination of the formation constants of complex compound was used. All the pH determinations were carried out with a Beckmann pH meter, model G, using a glass electrode and a reference calomel electrode. The concentration of the chelating agent was increased by titrating with carbonate-free caustic soda from a burette. During titration the solution containing the ligand and the boric acid was stirred vigorously by a magnetic stirrer. All the determinations were carried out in aqueous solution and at room temperature (28° C.).

Carbonate-free alkali.—A thick paste of sodium hydroxide was prepared and centrifuged. Any traces of carbonate present settled down at the bottom of the tube. This was then poured in a flask containing previously boiled distilled water. The caustic soda solution was then standardised against a standard acid and used for titration in all the experiments. It was protected from atmospheric carbon dioxide by putting a soda lime guard tube.

For preparing the ligands and the boric acid solution, the water was previously boiled to free it from carbon dioxide. To avoid decomposition ligands were not heated under any circumstance.

All the chemicals used were of E. Merck G.R. or B.D.H. AnalaR quality.

While calculating $\bar{n}$ and (A), account was taken of the dilution due to the addition of alkali during titrations.

The $\bar{n}$ and (A) values in titrations with catechol and gallic acid are given in Tables I and II respectively.

Discussion

Boron having the group valency three has one orbital free which will help it in complex formation to a considerable extent. In all the complexes reported, boron is quadri-covalent. The stability data for boron complexes in water solution are practically non-existent in literature. This is due to the preferential behaviour of the boron ion to attach itself more strongly to hydroxyl group in aqueous solution, rather than any other donor group.

TABLE I

Titration with catechol

k for catechol = $3 \cdot 3 \times 10^{-10}$; $t = 28°$ C.

($C_b = 0 \cdot 01$ mole/litre, $C_{ch} = 0 \cdot 098$ mole/litre, $C_{H^+} = 0 \cdot 1078$ M, NaOH = 2·02 N)

ml. of base	pH	(A)	$\bar{n}$
5·90	5·95	$2 \cdot 682 \times 10^{-5}$	0·144
5·95	6·15	$4 \cdot 205 \times 10^{-5}$	0·237
6·00	6·31	$6 \cdot 015 \times 10^{-5}$	0·330
6·10	6·72	$1 \cdot 512 \times 10^{-4}$	0·526
6·30	7·69	$1 \cdot 345 \times 10^{-3}$	0·808
6·50	8·12	$3 \cdot 454 \times 10^{-3}$	0·975

pK (from formation curve) = 3·89 (Fig. 4).

TABLE II

Titration with gallic acid

For gallic acid, $k_1 = 3 \cdot 98 \times 10^{-5}$ $t = 28°$ C.
$k_2 = 2 \cdot 51 \times 10^{-9}$

($C_b = 0 \cdot 01$ mole/litre, $C_{ch} = 0 \cdot 0588$ mole/litre, NaOH = 1·9 N)

ml. of base	pH	(A)	$\bar{n}$
3·20	6·28	$2 \cdot 566 \times 10^{-4}$	0·247
3·30	6·55	$4 \cdot 664 \times 10^{-4}$	0·378
3·35	6·71	$6 \cdot 658 \times 10^{-4}$	0·442
3·40	6·90	$1 \cdot 016 \times 10^{-3}$	0·490
3·45	7·05	$1 \cdot 416 \times 10^{-3}$	0·524
3·50	7·20	$1 \cdot 958 \times 10^{-3}$	0·577

pK (from formation curve) = 2·97 (Fig. 5).

Nevertheless, preparative chemistry has shown that boron forms well defined complexes of boric acid with one or two molecules of orthohydroxy organic acids or other orthohydroxy organic compounds.

Complex compounds of boron with salicylic acid and catechol were isolated by H. Schaefer.[3] All these ligands first form the monoligand boron complexes and then the diligand compounds. They were generally made by mixing equi-molar parts of boric acid and the complexing agent in aqueous solution and then evaporating almost to dryness, or by the addition of a large excess of the ligand and crystallising the compound from the syrupy solution.

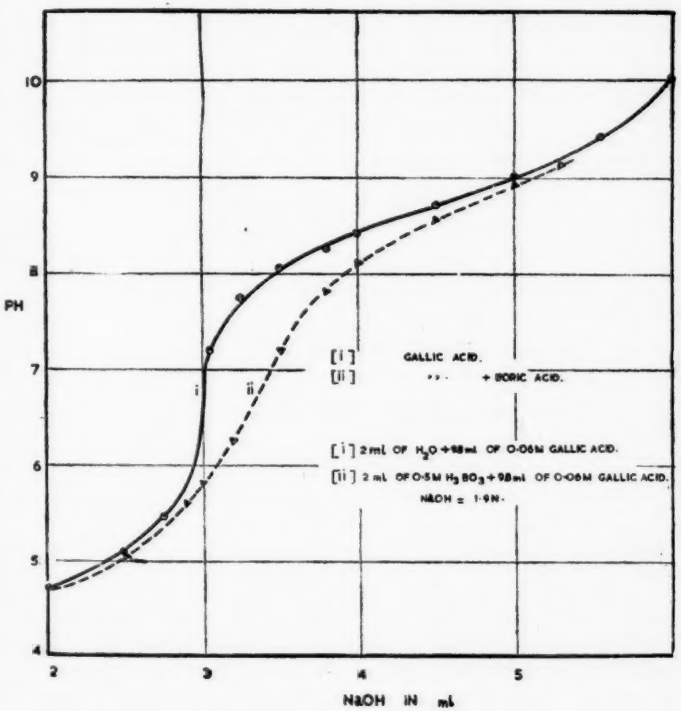


FIG. 1

Earlier studies on the boron complex compounds with ligands, such as mannitol (H. Schaefer[4]) and pyrocatechol (H. Schaefer[5]) have shown that the value of k_1 is generally of the order of 10^{-3} to 10^{-4}. The value of k_1 for

the salicylic acid complex has been reported to be of the order of 10^{-7} (H. Schaefer[6]).

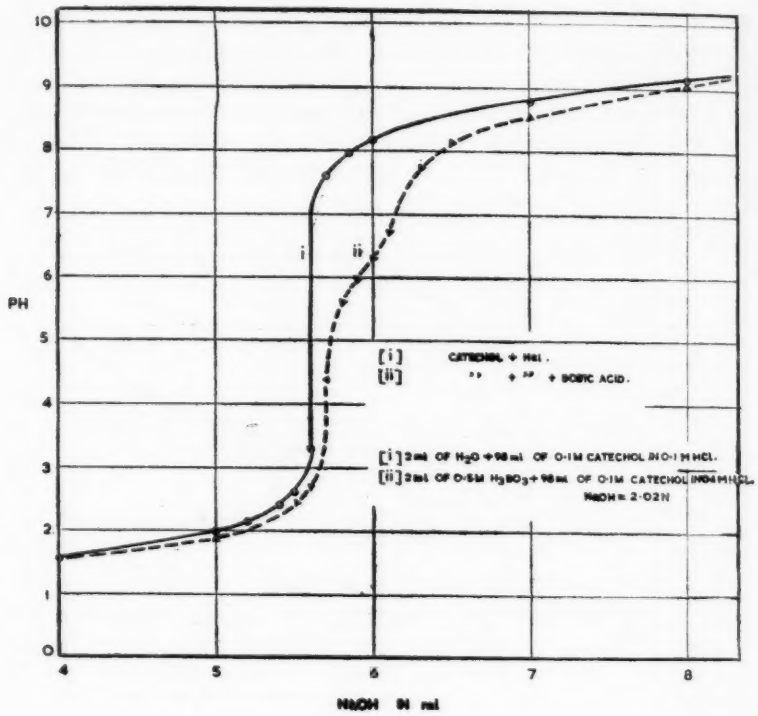

PH
NaOH N ml
i
ii
[i] CATECHOL + HCl.
[ii] " + " + BORIC ACID.
[i] 2 ml OF H_2O + 98 ml OF 0·1M CATECHOL IN 0·1 M HCl.
[ii] 2 ml OF 0·5M H_3BO_3 + 98 ml OF 0·1M CATECHOL IN 0·4 M HCl.
NaOH = 2·02 N

FIG. 2

The four hydroxy aromatic compounds used as ligands in the present investigation are gallic acid, catechol, resorcinol and salicylic acid. In all our experiments, the concentration of the ligands and boric acid was kept very low and the concentration is assumed as equal to activity. No foreign ions, such as Cl^- or NO_3^- were added during the titration to keep the ionic strength constant. Only with catechol a small amount of free hydrochloric acid was added. The values of formation constants reported here should not be taken as absolute values but just representing the order of magnitude.

With gallic acid a definite complex is formed as can be seen from the curve (Fig. 1). The calculation of the formation constant shows that the

value of k_1 is of the order of 10^{-3}, while k_2 is too small to be satisfactorily determined under our experimental conditions.

The curve with catechol (Fig. 2) also indicates a definite complex formation. Calculation of the formation constant by Bjerrum's method shows that k_1 is of the order of 10^{-4} which is in agreement with the value obtained by H. Schaefer.[4, 5]

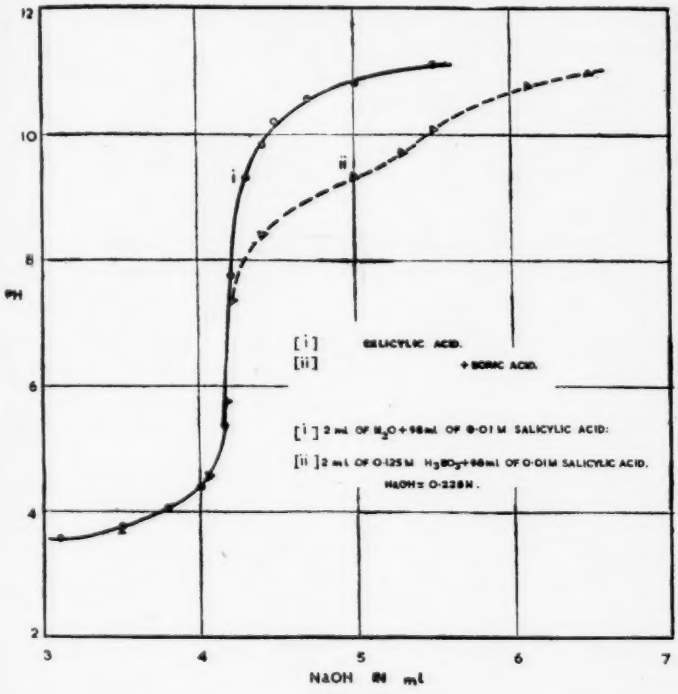


FIG. 3

The titration curve does not indicate any complex formation when resorcinol is used as ligand (Fig. 6). In the present investigation it has been found that salicylic acid does not form any complex compound with boric acid under the conditions of the experiment. The curve (Fig. 3) showing the titration of boric acid and salicylic acid shows a considerable deviation from the neutralisation curve of the salicylic acid alone which apparently indicates complex formation. But if we superimpose the boric acid titration curve on the neutralisation curve of the salicylic acid, the resultant curve will exactly be the same as the titration curve of the mixture of salicylic acid

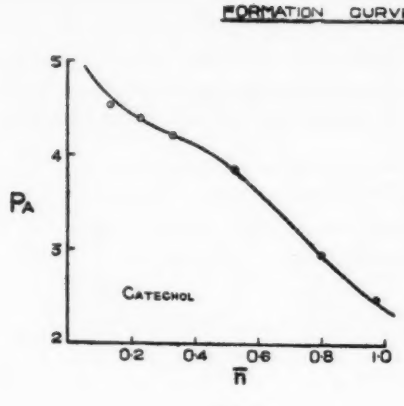


FIG. 4

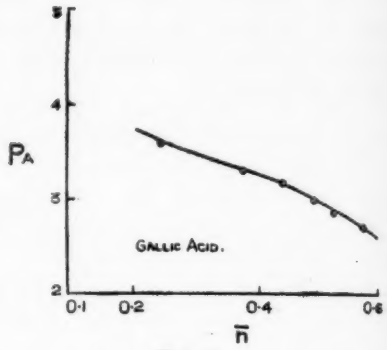


FIG. 5

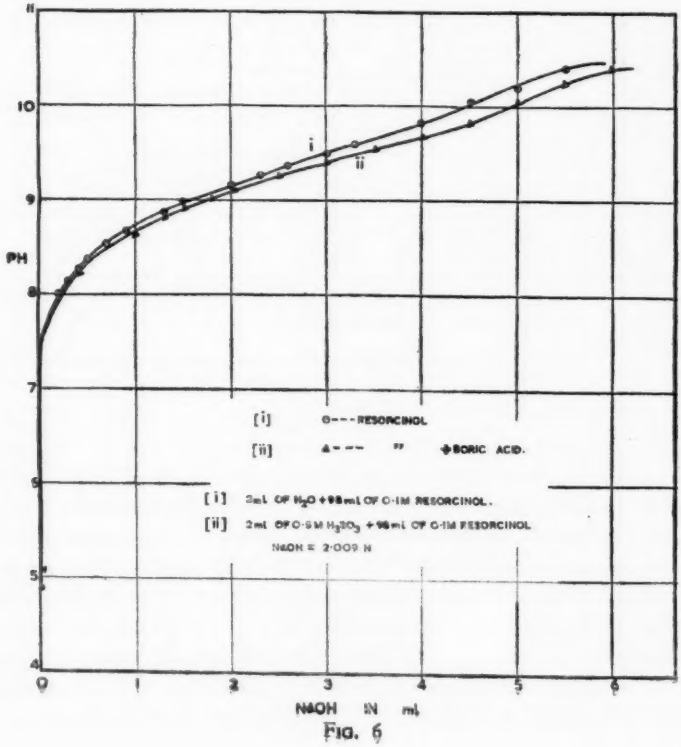


FIG. 6

and boric acid. So, it seems that there is actually no boron complex formed with salicylic acid under the experimental conditions used.

Schaefer,[6] however, had indicated that free boric acid does not have a great tendency to form complex with salicylic acid for the fact that the dissociation of the former is still further reduced due to the hydrogen ions from the latter.

$$H_3BO_3 \rightleftarrows H_2BO_3^- + H^+.$$

To overcome this difficulty, an attempt has been made to see if boron complex with salicylic acid can be formed by using boric acid partially neutralised with NaOH. However, the calculations do not show any sign of complex formation.

With mannitol it has been observed by various workers that higher the mannitol concentration the more the complex formation with boric acid till the concentration of mannitol becomes so large that its decrease during the titration is insignificant (H. Schaefer[4]). In the case of salicylic acid it is not possible to use a large excess of the ligand due to its low solubility in water. The present authors, however, used sodium salicylate and kept the concentration of boron to ligand as 1 : 90 but failed to notice any indication of complex formation.

From the above findings it is concluded that under the experimental conditions, while boron forms complexes with catechol and gallic acid, it does not show any tendency to complex formation with resorcinol and salicylic acid. From the k values, it is seen that the catechol complex with boron is stronger than that with gallic acid. It seems that the hydroxy groups in the ortho-position help complex formation with boron. On the other hand, the presence of carboxylic acid group either retards the complex formation or weakens the complex if formed, which may be attributed to the retardation of the ionization of boric acid in solution by H^+ ion.

ACKNOWLEDGEMENT

The authors' thanks are due to Dr. Jagdish Shankar for his keen interest during the course of the work.

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2. Maley, L. E. and Mellor, D.P. *Aust. J. of Sci. Res.*, 1949, **2 A**, 92.
3. Schaefer, H. .. *Z. anorg. allg. Chem.*, 1942, **250**, 82.
4. ——— .. *Ibid.*, 1941, **247**, 96.
5. ——— .. *Ibid.*, 1942, **250**, 127.
6. ——— .. *Ibid.*, 1942, **250**, 82.

A NEW SYNTHESIS OF ISLANDICIN AND CYNODONTIN

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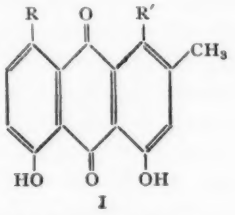
Abstract

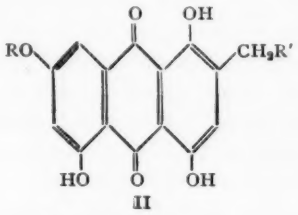
2-Methylanthraquinones with the 1 : 4-dihydroxy system are conveniently prepared by the persulphate oxidation of the intermediate benzoylbenzoic acids and subsequent ring closure. 2-Methylquinizarin, islandicin and cynodontin have been prepared by this method as typical examples.

Among the naturally occurring polyhydroxyanthraquinones of fungal origin,[1] the following have 1 : 4-dihydroxy system: helminthosporin (I *a*), islandicin (I *b*), cynodontin (I *c*), catenarin (II *a*), erythroglaucin (II *b*) and tritisporin (II *c*). For the synthesis of these compounds, a number of methods[2–4] have been used in the past but none of them follows any possible path of biogenesis.



I

(*a*) R=OH; R′=H
(*b*) R=H; R′=OH
(*c*) R=R′=OH



II

(*a*) R=R′=H
(*b*) R=CH₃; R′=H
(*c*) R=H; R′=OH

A possible scheme of evolution of lichen and mould anthraquinones has been recently outlined.[5] It is based on their origin from C_8-unit (orsellinic unit) and its modifications. Two such units are involved in the formation of mould anthraquinones. The above-mentioned compounds having the 1 : 4-dihydroxy system are considered to be related to simpler compounds and involve an extra stage of *para* nuclear oxidation. Based on this idea, the anthraquinones, catenarin (II *a*), erythroglaucin (II *b*) and tritisporin (II *c*) are related to *frangula*-emodin, physcion and ω-hydroxy-emodin respectively. Similarly, helminthosporin (I *a*) and islandicin (I *b*)

may be related to the common precursor, chrysophanol. Though they do not occur together, this biogenetical relationship between chrysophanol and islandicin is supported by the occurrence of the former in the place of the latter as a metabolic product of one of the strains (N.R.R.L. 1175) of *Penicillium islandicum* Sopp., examined by Howard and Raistrick.[6] Cynodontin (I *c*) then involves an extra stage of nuclear oxidation as compared with helminthosporin (I *a*) or islandicin (I *b*) and this view is supported by the occurrence of helminthosporin (I *a*) and cynodontin (I *c*) together in the metabolic product of *Helminthosporium cynodontis* Marignoni.[7]

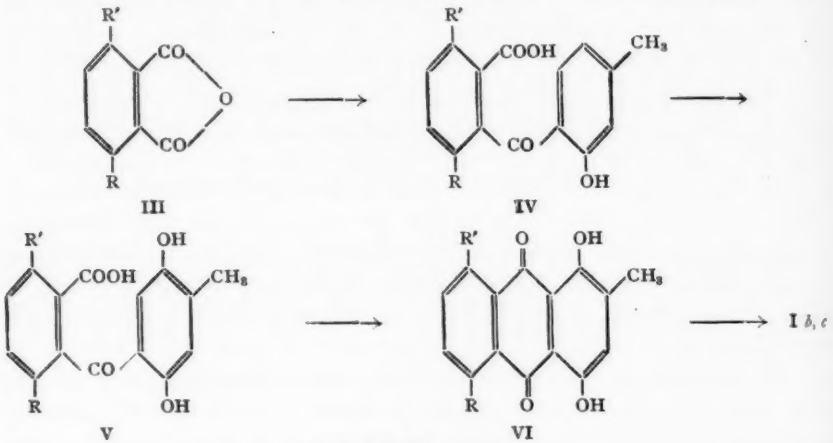
Experiments carried out in the past for the direct nuclear oxidation of hydroxyanthraquinones under mild conditions using persulphate have not been successful.[8-9] It could be inferred that in Nature too, this oxidation is not feasible. This result could be attributed to lack of adequate reactivity of the nuclear positions. Consequently, the alternative possibility of oxidation in an earlier stage such as the benzoylbenzoic acid stage or even at the C_6-unit stage had to be considered. The feasibility of the *para* oxidation of the hydroxybenzoylbenzoic acids has now been investigated using simpler examples.

The simplest example of this series would be 2-methylquinizarin though it has not, so far, been found to occur in Nature. 2-(2′-Hydroxy-4′-methyl) benzoylbenzoic acid (IV *a*), obtained by the condensation of phthalic anhydride (III *a*) and *m*-cresol, has now been subjected to oxidation with potassium persulphate in alkaline medium. The resulting quinol derivative (V *a*) has been cyclised with concentrated sulphuric acid when 2-methylquinizarin (VI *a*) is obtained. This was earlier prepared by Ullmann and Schmidt[10] by a different method.

A more complex member is islandicin (I *b*). For its synthesis, 3-methoxyphthalic anhydride (III *b*) is condensed with *m*-cresol to yield 3-methoxy-2-(2′-hydroxy-4′-methyl) benzoylbenzoic acid (IV *b*) which undergoes smooth oxidation to yield the quinol derivative (V *b*). Subsequent ring closure could be effected using fuming sulphuric acid and melted boric acid at 70° yielding 1 : 4-dihydroxy-5-methoxy-2-methylanthraquinone (VI *b*). Demethylation of this substance with hydrobromic acid-acetic acid mixture gives islandicin (I *b*) which is found to be identical with the natural sample.[11] The use of sulphuric acid alone at a higher temperature produces ring closure as well as demethylation but the yield is found to be poor.

The mould product, cynodontin (I *c*) has a hydroxyl group more than islandicin (I *b*). Its synthesis has now been carried out using 3 : 6-dimethoxyphthalic anhydride, prepared by a modification of the method of Graves

and Adams.[12] A parallel series of experiments yielded the benzoylbenzoic acid (IV *c*) which gave the corresponding quinol derivative (V *c*) by oxidation. Ring closure to the corresponding dihydroxy-dimethoxy-anthraquinone (VI *c*) and subsequent demethylation yielded 1 : 4 : 5 : 8-tetrahydroxy-2-methylanthraquinone (I *c*) which is identical with a sample of cynodontin, isolated by Raistrick *et al.*[7] as a metabolic product of *H. cynodontis* Marignoni.



(*a*) R=R'=H
(*b*) R=OCH_3; R'=H
(*c*) R=R'=OCH_3

EXPERIMENTAL

2-(2' : 5'-Dihydroxy-4'-methyl) benzoylbenzoic acid (*V* a).—To a continuously stirred solution of 2-(2'-hydroxy-4'-methyl) benzoylbenzoic acid[10] (2·5 g.) in aqueous potassium hydroxide (3 g. in 30 c.c.) was added, dropwise in the course of 2 hrs., a saturated solution of potassium persulphate (3 g.) in water (18–20°). The deep brown solution was kept overnight at laboratory temperature and then acidified (Congo red) and the unreacted acid (0·5 g.) was extracted with ether. Concentrated hydrochloric acid (20 c.c.) and sodium sulphite (3 g.) were added to the aqueous solution, and the mixture heated on a boiling water-bath for half an hour and then cooled in the refrigerator when a crystalline solid separated. It was filtered and crystallised from water when the dihydroxy acid was obtained as orange-yellow prisms, m.p. 161–62.° Yield, 0·5 g. (Found: C, 65·7; H, 4·1; $C_{15}H_{12}O_5$ requires C, 66·2; H, 4·4%). It gave a red solution with aqueous sodium hydroxide and a green colour with alcoholic ferric chloride changing to brown with excess of the reagent.

1 : 4-*Dihydroxy*-2-*methylanthraquinone* (2-*methylquinizarin*) (*VI* a).—The above oxidation product (0·5 g.) was heated with concentrated sulphuric acid (3 c.c.) in an oil-bath at 150° for ½ hr., and the deeply coloured solution carefully diluted with water when a scarlet-red precipitate separated. It was extracted with chloroform and the chloroform solution dried over anhydrous sodium sulphate. On removal of the solvent and crystallisation of the residue from methanol or dioxan containing a little water, 2-methylquinizarin was obtained as scarlet needles, m.p. 176–77°. Yield, 0·3 g. It gave a greenish brown colour with alcoholic ferric chloride and a violet colour with aqueous sodium hydroxide. Its solution with concentrated sulphuric acid was pink-violet and the solution in organic solvents was red with a green fluorescence. With methanolic magnesium acetate it gave a purple colour. Ullmann and Schmidt[10] also reported the same m.p. for 2-methylquinizarin.

Its acetate was prepared using acetic anhydride and concentrated sulphuric acid and it crystallised from ethyl acetate as yellow prisms, m.p. 208–10° (Found: C, 67·0; H, 4·5; $C_{19}H_{14}O_6$ requires C, 67·5; H, 4·2%). It did not give any colour with alcoholic ferric chloride. Nietzki[13] reported 185° as the m.p. of the acetate of 2-methylquinizarin.

3-*Methoxy*-2-(2′-*hydroxy*-4′-*methyl*) *benzoylbenzoic acid* (*IV* b).—3-Methoxyphthalic anhydride[14] (III *b*) (5 g.) was dissolved in warm *m*-cresol (30 c.c.) and to the cooled solution, powdered anhydrous aluminium chloride (10 g.) was added in small lots. The reaction mixture was kept overnight at room temperature and then heated at 70° for 6 hrs. with mechanical stirring. The dark red reaction mixture was allowed to stand overnight and then treated with ice and concentrated hydrochloric acid (10 c.c.). The excess of cresol was steam distilled and the supernatant aqueous layer decanted when the residual oil quickly solidified. It was dissolved in 1 *N* sodium hydroxide (150 c.c.) and the solution saturated with carbon dioxide. The dull yellow phthalein by-product, which separated, was filtered and washed with water. The filtrate was acidified with ice-cold hydrochloric acid when the benzoylbenzoic acid separated as a brown solid. It was crystallised from methanol when the acid was obtained as colourless prisms, m.p. 210–12°. It gave a red colour with alcoholic ferric chloride and was soluble in aqueous sodium bicarbonate. Yield, 1·2 g. (Found: C, 66·9; H, 4·8; $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%).

3-*Methoxy*-2-(2′: 5′-*dihydroxy*-4′-*methyl*) *benzoylbenzoic acid* (*V* b).—A solution of the above benzoylbenzoic acid (IV *b*) (1 g.) in aqueous potassium hydroxide (1·1 g. in 25 c.c. water) was treated with a solution of potassium

persulphate (1·2 g.) and was worked up as described earlier. The nuclear oxidation product crystallised from methanol as pale yellow tiny prisms, m.p. 228–31° (decomp.). Yield, 0·5 g. (Found: C, 63·1; H, 4·9; $C_{16}H_{14}O_6$ requires C, 63·6; H, 4·7%). It gave a green colour with alcoholic ferric chloride which changed to brown on shaking in air or with excess of the reagent. It was soluble in aqueous sodium bicarbonate.

1 : 4-*Dihydroxy*-5-*methoxy*-2-*methylanthraquinone* (*VI* b).—The above acid (V *b*) (0·2 g.) was heated with a mixture of melted and finely powdered boric acid (0·2 g.) and sulphuric acid monohydrate (3 c.c.) on a water-bath. At 70°, fuming sulphuric acid (0·5 c.c.) was added and the mixture allowed to stand for an hour at that temperature. The deep reddish blue solution was then poured on crushed ice and stirred well. After a few hours, the precipitate was coagulated by heating on a boiling water-bath. The red solid was filtered, washed with hot water and dried. It crystallised from chloroform-methanol mixture as long bright red needles, m.p. 197–99°. Yield, 0·15 g. (Found: OCH_3, 10·1; $C_{16}H_{12}O_5$ requires 1 OCH_2, 10·9%).

It was insoluble in aqueous sodium bicarbonate and sodium carbonate but readily soluble in aqueous sodium hydroxide giving a deep violet solution from which a violet precipitate separated on standing. It gave a purple colour with methanolic magnesium acetate. It was soluble in glacial acetic acid giving an orange-yellow solution with a green fluorescence. With concentrated sulphuric acid, it gave a purple-red colour in bulk and bluish red in thin layers with a fiery red fluorescence.

1 : 4 : 5-*Trihydroxy*-2-*methylanthraquinone* (*islandicin*) (*I* b).—The above methyl ether (VI *b*) (0·1 g.) was refluxed with a mixture of glacial acetic acid (10 c.c.) and hydrobromic acid (constant boiling; 15 c.c.) for 5 hrs. The hot reaction mixture was filtered and cooled in ice, when glistening red plates separated which were filtered and washed well with water. The solid was dried and crystallised from either chloroform or dioxan when islandicin was obtained as dark red lustrous large plates or leaflets, m.p. 216–18°. Yield, 0·085 g. (Found: C, 67·1; H, 3·8; $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%).

It was insoluble in aqueous sodium bicarbonate and carbonate but readily soluble in aqueous sodium hydroxide giving a violet solution the colour of which faded to pale yellow after a few hours' exposure to air. With cold concentrated sulphuric acid, it gave a bright purple-red colour in bulk and bluish red in thin layers with a fiery red fluorescence. Its solution in glacial acetic acid was yellow-orange in colour with a green fluorescence.

With methanolic magnesium acetate, it gave a purple colour with a violet fluorescence. Mixed m.p. with the natural sample[11] was undepressed.

The above synthetic sample of islandicin was acetylated using acetic anhydride containing a drop of concentrated sulphuric acid. The acetate crystallised from methanol as pale yellow needles, m.p. 206–08° alone or when mixed with the acetate prepared from the natural sample of islandicin.

The methyl ether, prepared by the dimethyl sulphate-acetone-potassium carbonate method, crystallised from aqueous methanol as orange-yellow prisms, m.p. 159–60° (lit.,[11] m.p. 161°).

2:3-*Dicyanohydroquinone dimethyl ether*.—The following method was found to be more convenient than the earlier methods.[12, 15]

2:3-Dicyanohydroquinone[16] (20 g.), dissolved in dry acetone (400 c.c.), was refluxed with potassium carbonate (80 g.) and dimethyl sulphate (18 c.c., 3 moles.) for 4 hrs. The methyl ether began to separate in about 15 minutes. The reaction mixture was filtered and the residue containing the methyl ether and the potassium salts was treated with water to dissolve the latter. The insoluble solid was filtered and washed with water and then with a little acetone. It crystallised from glacial acetic acid as colourless needles, m.p. 280–81°. Yield, 21 g. (lit.,[12] m.p. 275°).

3:6-*Dimethoxy*-2-(2′:5′-*dihydroxy*-4′-*methyl*) *benzoylbenzoic acid* (*V* c).—3:6-Dimethoxyphthalic anhydride required for this was obtained by the hydrolysis of the above dimethyl ether.[12]

A solution of 3:6-dimethoxy-2-(2′-hydroxy-4′-methyl) benzoylbenzoic acid[12] (IV *c*) (0·9 g.) in potassium hydroxide (0·7 g. in 17 c.c. of water) was treated with a saturated solution of potassium persulphate (1 g.) and worked up as described earlier. The quinol derivative crystallised from aqueous methanol as clusters of yellow prisms, m.p. 227–29° (decomp.). Yield, 0·4 g. (Found: C, 60·9; H, 5·1; $C_{17}H_{16}O_7$ requires C, 61·4; H, 4·9%). It gave a green colour with alcoholic ferric chloride which turned brown on keeping or on addition of excess of the reagent. Mixed m.p. with the starting material (m.p. 231–33°) was depressed (216–20°).

1:4-*Dihydroxy*-5:8-*dimethoxy*-2-*methylanthraquinone* (*VI* c).—The above benzoylbenzoic acid (0·25 g.) was cyclised with boric acid (0·25 g.), sulphuric acid monohydrate (7 c.c.) and fuming sulphuric acid (0·3 c.c.) as in the case of islandicin. The anthraquinone derivative crystallised from chloroform-methanol mixture as red needles, m.p. 222–24°. Yield, 0·15 g. (Found: C, 64·6; H, 4·8; OCH_3, 18·8; $C_{17}H_{14}O_6$ requires C, 64·9; H, 4·5; $2\,OCH_3$, 19·7%).

It was insoluble in aqueous sodium bicarbonate and carbonate solutions, but soluble in alkali giving a blue-violet solution. With concentrated sulphuric acid, it gave a deep blue colour and with methanolic magnesium acetate a bluish violet colour.

1 : 4 : 5 : 8-*Tetrahydroxy-2-methylanthraquinone* (*cynodontin*) (*I* c).—The above methyl ether (0·12 g.) was demethylated using glacial acetic acid (10 c.c.) and hydrobromic acid (constant boiling; 20 c.c.) as described earlier. Cynodontin crystallised from chloroform as brown leaflets with a bronze lustre, m.p. 258–60° alone or when mixed with the natural sample.[7] Yield, 0·05 g. (Found: C, 63·0; H, 4·0; $C_{15}H_{10}O_6$ requires C, 62·9; H, 3·5%). It was identical in properties and colour reactions with the natural sample of cynodontin. It gave a blue colour with methanolic magnesium acetate.

We convey our thanks to Professors H. Raistrick and J. H. Birkinshaw for the gift of the natural samples of islandicin and cynodontin for comparison.

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CONSTITUTION OF VOGELETIN

The Pigment from the Seeds of *Tephrosia vogelii* Hook.

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The seeds of *Tephrosia vogelii* Hook. (Fam.: Leguminosæ) grown in the Nilgiris, South India, were examined by Rangaswami and Sastry.[1] Besides the rotenoid compounds tephrosin, deguelin and dehydrodeguelin, they reported the isolation of a new substance which answered some of the colour reactions characteristic of the anthoxanthin group of compounds. Chemical work on the constitution of this substance, which is now given the name vogeletin, is described in this paper.

On the basis of the several degradation and other reactions described in the sequel, the molecular formula of vogeletin is now revised to $C_{16}H_{12}O_7$. It is soluble in alkali and gives a deep green colour with alcoholic ferric chloride and an orange precipitate with lead acetate. It contains a methoxyl group and is free from methylenedioxy groups. It gives a pink colour on reduction with magnesium and hydrochloric acid, zinc and hydrochloric acid or sodium amalgam. It gives a positive reaction in Wilson's boric acid test.[2] These reactions indicate that vogeletin may be a hydroxy flavone or flavonol with free hydroxyl groups and a methoxyl group and that position 5 is probably occupied by a hydroxyl or a methoxyl.

Vogeletin forms a tetracetate with acetic anhydride and sodium acetate and a tetramethyl ether with dimethyl sulphate and potassium carbonate indicating that it is a tetrahydroxy compound. Since the same methyl ether was produced even when diazomethane was employed as the methylating agent, it can be concluded that there is no specially resistant hydroxyl group. This point is referred to again in the sequel.

Information regarding the positions of the substituent groups was obtained from alkaline fission experiments. Vogeletin itself proved resistant to the mild alkaline conditions employed by us, but O-tetramethylvogeletin underwent smooth cleavage. One of the fission products was anisic acid (IV), which proved that the side phenyl nucleus of the anthoxanthin carries only one methoxyl group and that it is in the 4′-position. The other fission product answered the colour reactions of an ortho-hydroxy

aromatic ketone. Its analysis corresponds to $C_{12}H_{16}O_6$ with 4 methoxyl groups (III). It was characterised as its 2:4-dinitrophenylhydrazone and it gave a monomethyl ether which was a liquid and was eventually characterised as its 2:4-dinitrophenylhydrazone.

The positions of the substituent groups in the benzopyrone part of the molecule could be deduced from a combination of demethylation experiments and colour reaction studies. Tetramethyl vogeletin underwent complete demethylation with boiling hydriodic acid to give a pentahydroxyflavone (V). The same compound was obtained from vogeletin by the action of hydriodic acid. This compound (V) which may be called *nor*-vogeletin was characterised as its penta-acetate and it could be reconverted to tetramethylvogeletin by methylation with dimethyl sulphate and potassium carbonate. Compound (V) answered the Bargellini's test for 5, 6, 7-trihydroxyflavones,[3] but the gossypetone reaction was negative indicating the absence of a 5:8-hydroxy combination.[4] Thus the benzopyrone part in vogeletin, its *nor*-compound and its tetramethyl ether should carry oxygen functions in positions 5, 6 and 7. The only position now left for the remaining oxygen is position 3 of the benzopyrone ring. Thus tetramethyl vogeletin should be 3, 5, 6, 7, 4′-pentamethoxyflavone (II); *nor*-vogeletin should be 3, 5, 6, 7, 4′-pentahydroxyflavone (V); and the phenolic ketone should be 2-hydroxy-ω, 4, 5, 6-tetramethoxy-acetophenone (III). Structure V for the pentahydroxy compound and II for the complete methyl ether are compatible with the observation that the product of demethylation (VI) of II with anhydrous aluminium chloride is identical with the product of methylation of V with diazomethane; in the former reaction aluminium chloride selectively demethylates the methoxyl in the 5 position, and in the latter reaction diazomethane methylates all the hydroxyls except the one in the 5-position. The melting-points and colour reactions of V and III agree with those described in the literature[5–6] for compounds having these structures, and the melting-points of II and VI agree with those recorded in the literature.[5–7] A reference sample of 6-hydroxy-3, 5, 7, 4′-tetramethoxyflavone, kindly supplied by Prof. T. R. Seshadri of the Delhi University, was methylated with dimethyl sulphate and potassium carbonate in acetone solution. The pentamethoxy compound thus obtained and compound II obtained from vogeletin had the same melting-point, both separately and in admixture. Structure III for the phenolic ketone also agrees with our observation that it couples with diazotised sulphanilic acid to yield a coloured dye.

The position of the single methoxyl group (and the four hydroxyl groups) in vogeletin itself could be deduced from the following considerations: When vogeletin was ethylated with diethyl sulphate and potassium carbonate it yielded a tetraethyl ether (VII) which on alkaline fission gave an acid identified as *p*-ethoxybenzoic acid (VIII). This proves the existence of a free hydroxyl group in position 4′ in vogeletin. In contrast to *nor*-vogeletin (V), vogeletin does not give a positive Bargellini test but it reacts with methylene iodide in the presence of potassium carbonate to give a compound (IX) which contains the methylenedioxy group besides a methoxyl group. This would lead to one of the two alternative combinations, 5-methoxy-6, 7-dihydroxy or 5, 6-dihydroxy-7-methoxy in the benzene ring of the benzopyrone part. Since both dimethyl sulphate and diazomethane react with vogeletin to give the same complete methyl ether (II), position 5 should be already having the methoxyl group and vogeletin should be given the structure 5-methoxy-3, 6, 7, 4′-tetrahydroxyflavone (I). This accords well with its colour reactions. The several transformations mentioned above are represented in the scheme given on page 244.

The occurrence of natural flavonoids carrying a methoxyl group in the 5-position is not very common. There are a few instances where all the hydroxyls including the 5 are methylated, *e.g.*, nobiletin (5, 6, 7, 8, 3′, 4′-hexamethoxyflavone),[8] tangeretin (5, 6, 7, 8, 4′-pentamethoxyflavone),[9–10] meliternatin (3, 5, 7, 8-tetramethoxy-3′-4′-methylenedioxyflavone).[11] But the occurrence of the 5-hydroxyl group in the methylated condition, when hydroxyl groups in the more reactive positions occur free, is something extraordinary. Known instances of this type are azaleatin (5-methoxy-3, 7, 3′, 4′-tetrahydroxyflavone),[12] muningin (5, 7-dimethoxy-6, 4′-dihydroxy-isoflavone)[13] and isopedicin (5, 7, 8-trimethoxy-6-hydroxyflavanone).[14] To explain this it has been suggested by Seshadri[15] that methylation of the concerned hydroxy group occurs before the pyrone ring closes up and when the reactivity of the specific hydroxyl group is still unhindered.

Experimental

The coarsely powdered seeds (20 kg.) were defatted with petroleum ether (4×12 litres). The marc was air-dried, powdered again and extracted with cold methylated spirit (4×12 litres). The alcohol extract was concentrated (to 2 litres) and poured into excess of water (6 litres) and extracted with chloroform to remove the rotenoid compounds and the resins. The clear aqueous liquid having a red colour was concentrated in vacuum (to 2 litres) and the glycoside of the flavone hydrolysed by heating with 7% sulphuric acid. The pigment separated out as a black granular residue

IX
I
II
VII
VIII
V
VI
IV
III
HI
CH_2N_2
CH_2I_2
Me_2SO_4 or CH_2N_2
Et_2SO_4
Alkali
$AlCl_3$
Me_2SO_4
+ HOOC—

which was filtered, washed with water and dried. Crystallization from acetone gave vogeletin as pale yellow needles, m.p. 283-84° (yield 9·0 g.) [Found: C, 61·2; H, 4·3; $-OCH_3$, 9·5. $C_{16}H_{12}O_7$ requires: C, 60·8; H, 3·8; $-OCH_3$ (1), 9·8%].

Vogeletin tetraacetate

Vogeletin (100 mg.) was boiled with acetic anhydride (2 c.c.) and fused sodium acetate (0·4 g.) for 3 hours in an oil-bath at 145° C. The reaction mixture was poured into water and allowed to stand overnight. The solid was filtered, washed with water and dried. Crystallization from alcohol gave colourless woolly needles, m.p. 211–12° [Found: C, 59·5; H, 4·5; $-COCH_3$, 34·1; $C_{24}H_{20}O_{11}$ requires: C, 59·5; H, 4·2; $-COCH_3$ (4), 35·6%].

Nor-*vogeletin* (*V*)

Vogeletin (200 mg.) was dissolved in acetic anhydride (5 c.c.) by heating. It was cooled and treated with hydriodic acid (*d*, 1·7; 10 c.c.). The mixture was heated in an oil-bath at 150–60° for 1 hour. It was then cooled, diluted with water and the solution decolourised by passing sulphur dioxide. The reddish yellow solid was filtered, washed repeatedly with hot water, dried and crystallized from alcohol. The *nor*-compound is a yellow substance assuming a slight green colour on exposure to air; m.p. is above 320° with darkening and without completely melting. It gives a bluish green ferric colour. Yield 100 mg. (Found: C, 59·7; H, 3·8; $-OCH_3$, nil. $C_{15}H_{10}O_7$ requires: C, 59·6; H, 3·3%).

Nor-*vogeletin penta-acetate*

Nor-vogeletin (100 mg.) was acetylated with acetic anhydride (3 c.c.) and fused sodium acetate (350 mg.) in the same way as vogeletin. The acetate crystallized from alcohol as colourless needles, m.p. 228–31° (Found: C, 59·1; H, 4·4. $C_{25}H_{20}O_{12}$ requires: C, 58·6; H, 3·9%).

O-*Tetramethylvogeletin* (*II*)

(*a*) *With dimethyl sulphate and potassium carbonate.*—Vogeletin (500 mg.) in acetone (100 c.c.) was treated with dimethyl sulphate (5 c.c.) and anhydrous potassium carbonate (15 g.) and the mixture refluxed on a water-bath for 12 hours. It was filtered, acetone removed from the filtrate and the residue treated with cold water (200 c.c.), stirred well and left in the ice-chest overnight. The solid was filtered, washed with water and dried. It was crystallized from alcohol and then from benzene-petroleum ether, when colourless needles were obtained, m.p. 152–53°. Mixed m.p. with authentic

sample of 3, 5, 6, 7, 4′-pentamethoxyflavone obtained by methylating 3, 5, 7, 4′-tetramethoxy-6-hydroxyflavone with dimethyl sulphate and potassium carbonate was undepressed [Found: C, 64·8; H, 6·0; $-OCH_3$, 39·7. $C_{20}H_{20}O_7$ requires: C, 64·5; H, 5·4; $-OCH_3$ (5), 41·7%].

(*b*) *With diazomethane.*—Vogeletin (100 mg.) was dispersed in absolute methanol (50 c.c.) and treated with excess of diazomethane in ether. The solution assumed a red colour and the substance slowly went into solution. After leaving overnight in the ice-chest, the solution was filtered, the solvents were removed from the filtrate and the residue was crystallized from benzene-petroleum ether. Colourless needles of the methyl ether were obtained, m.p. 152–53°. Mixed m.p. with the product obtained by method (*a*) was undepressed.

Methylation of nor-*vogeletin*

(*a*) *With dimethyl sulphate and potassium carbonate: Formation of compound II.—Nor*-vogeletin (50 mg.) dissolved in acetone (15 c.c.) was methylated with dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (1·5 g.) in the same way as vogeletin. The product obtained (50 mg.) melted at 152–53°, mixed m.p. with the tetramethyl ether of vogeletin was undepressed.

(*b*) *With diazomethane: Formation of compound VI.—Nor*-vogeletin (50 mg.) dissolved in absolute methanol (30 c.c.) was methylated by diazomethane in the same way as vogeletin. The product crystallized from alcohol as yellow prisms melting at 170–72°. Mixed m.p. with the compound obtained from vogeletin methyl ether (II) by the action of aluminium chloride (described below) was undepressed.

Demethylation of tetramethylvogeletin

(*a*) *With hydriodic acid: Formation of compound V.*—The methyl ether (200 mg.) dissolved in acetic anhydride (5 c.c.) was demethylated with hydriodic acid (*d*, 1·7; 10 c.c.) in the same way as vogeletin. Crystallization from alcohol yielded a yellow substance, m.p. above 320° with darkening and without completely melting. Colour reactions were the same as those of the compound obtained from vogeletin by the action of hydriodic acid. The mixed m.p., however, could not be taken since it was too high with charring and incomplete melting.

(*b*) *With anhydrous aluminium chloride*: *Formation of compound VI.*—The methyl ether (100 mg.) in absolute ether (40 c.c.) was treated with anhydrous aluminium chloride (2 g.) and the mixture shaken for ten minutes

and left overnight. The ether was evap orated o ff and the aluminium chloride complex was broken by treating with ice-cold hydrochloric acid and subsequent warming for half an hour. The substance was filtered, washed with water, dried and crystallized from alcohol as yellow prisms, m.p. 171–72° (Yield, 60 mg.) [Found: C, 64·2; H, 5·6; $-OCH_3$, 32·6. $C_{19}H_{18}O_7$ requires: C, 63·7; H, 5·1; $-OCH_3$ (4), 34·6%].

Tetraethyl vogeletin (*VII*)

Vogeletin (500 mg.) in acetone (100 c.c.) was refluxed for 12 hours with diethyl sulphate (7 c.c.) and anhydrous potassium carbonate (15 g.). After filtering the potassium salts and removing the solvent from the filtrate, a brown red liquid was obtained which remained unchanged on addition of water and leaving in the ice-chest for a day. It was taken into chloroform solution by extracting the mixture with the solvent. The chloroform solution was dried over anhydrous sodium sulphate, filtered and chloroform removed from the filtrate. The brown red liquid remaining was chromatographed on activated alumina (40 g.) employing for elution petroleum ether, benzene, chloroform and mixtures of these in the usual manner. The unchanged diethyl sulphate came down in the first two petroleum ether eluates and the ethyl ether in the subsequent eluates. The fractions showing uniform melting-point were mixed and recrystallized from ether-petroleum ether. The ethyl ether was obtained as colourless prisms, m.p. 84–85° (Yield, 450 mg.) [Found: C, 67·8; H, 6·9; $-OC_2H_5$, 51·1. $C_{24}H_{28}O_7$ requires: C, 67·3; H, 6·6; $-OC_2H_5$ (5), 52·6%, all alkoxyls being calculated as ethoxyls].

Methylenation of vogeletin: Formation of compound IX.—Vogeletin (200 mg.), acetone (20 c.c.), anhydrous potassium carbonate (0·5 g.) and methylene iodide (0·15 c.c.) were refluxed on a water-bath for 18 hours. The acetone was removed, water added and left overnight. It was filtered, washed, dried and crystallized from alcohol, when the methylenedioxy derivative (IX) separated as a reddish brown micro-crystalline substance melting at 272–76° (Yield, 35 mg.). It gave a green ferric colour, and a green colour on warming with gallic acid in concentrated sulphuric acid [Found: C, 61·7; H, 4·5; $-OCH_3$, 8·6. $C_{17}H_{12}O_7$ requires: C, 62·2; H, 3·7; $-OCH_3$ (1), 9·5%].

Alkali fission of tetramethylvogeletin (*II*)

Tetramethylvogeletin (1 g.) was treated with potassium hydroxide (2·0 g.) in absolute alcohol (40 c.c.) and the mixture refluxed on a water-bath for

6 hours. The solution was cooled, diluted with water and acidified with dilute hydrochloric acid (1 : 1). The alcohol was removed and the residue extracted with ether. The ether extract was fractionated into sodium bicarbonate-soluble, sodium hydroxide-soluble and neutral fractions.

The acid fraction (400 mg.) crystallized from alcohol as colourless prisms, m.p. 183–84° (IV) (Found: C, 63·6; H, 5·8. Anisic acid requires: C, 63·2; H, 5·3%). Mixed m.p. of IV with authentic sample of anisic acid was undepressed.

The phenolic fraction (560 mg.) was obtained as a reddish brown liquid which solidified after a few days. Crystallization from benzene-petroleum ether gave colourless woolly needles melting at 71–72°. It gave an olive green colour with alcoholic ferric chloride [Found: C, 56·8; H, 6·7; $-OCH_3$, 46·9. $C_{12}H_{16}O_6$ (compound III) requires: C, 56·3; H, 6·3; $-OCH_3$ (4), 48·4%].

The neutral fraction (10 mg.) did not give any crystalline substance.

The 2: 4-dinitrophenylhydrazone of the above phenolic ketone (III) prepared in the usual way was obtained as orange woolly needles, m.p. 179–83° (Found: C, 49·7; H, 5·2; $-OCH_3$, 26·9; N, 12·8. $C_{18}H_{20}O_9N_4$ requires: C, 49·6; H, 4·6; $-OCH_3$ (4), 28·4; N, 12·8%).

The methyl ether of phenolic ketone (III) was prepared using dimethyl sulphate and anhydrous potassium carbonate in acetone solution. The product was worked out in the usual manner when a brown coloured liquid was obtained which did not solidify. Purification by passing an acetone solution of it over a small column of alumina did not yield a solid product. It was converted in the usual way into the 2: 4-dinitrophenylhydrazone which crystallised from alcohol as red prisms, m.p. 168–70° [Found: $-OCH_3$, 34·6. $C_{19}H_{22}O_9N_4$ requires: $-OCH_3$ (5), 34·4%].

Alkali fission of tetraethylvogeletin (VII)

The ethyl ether (350 mg.) was refluxed with an absolute alcoholic potassium hydroxide solution (5%, 20 c.c.) for 6 hours and the products were worked up as in the case of the fission of the methyl ether (II). The acid fraction (VIII) (70 mg.) was recrystallized from alcohol, when it was obtained as colourless prisms, m.p. 194–95° (Found: C, 65·4; H, 6·5. *p*-Ethoxy benzoic acid requires: C, 65·0; H, 6·1%). Mixed m.p. of the above product (VIII) with *p*-ethoxybenzoic acid prepared according to the method of Cohen and Dudley[16] was undepressed. The phenolic fraction was a thick red liquid which did not solidify and was not examined further.

Summary

The constitution of the pigment isolated from the seeds of *Tephrosia vogelii* Hook. (now designated as vogeletin) has been established, by a study of its derivatives and by chemical degradation, as 5-methoxy-3, 6, 7, 4′-tetrahydroxyflavone.

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